Agilent
Trusted Answers

Agilent Automated Electrophoresis Portfolio

Sample Quality Control in Next-Generation Sequencing Workflows

Application Compendium

Agilent Automated Electrophoresis Portfolio

Sample Quality Control in Next-Generation Sequencing Workflows

Application Compendium

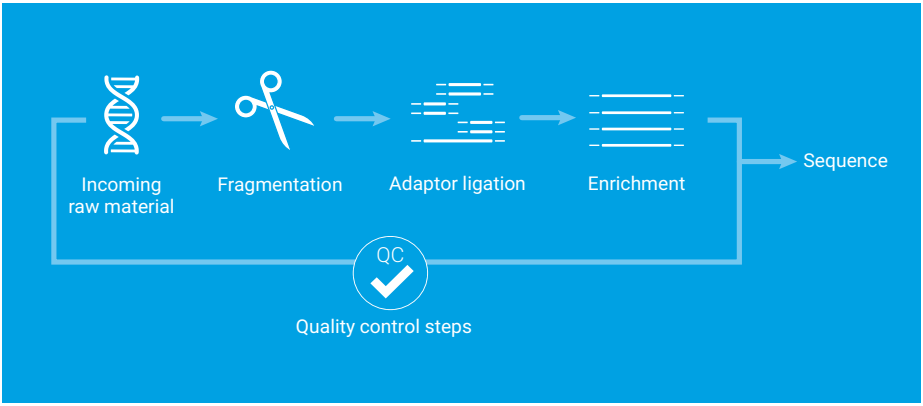
Contents

Contents

Sample Quality Control in Next-Generation Sequencing Workflows

Next-generation sequencing (NGS) is an essential tool in molecular biology laboratories for the analysis of nucleic acid samples in numerous basic, translational, and clinical research settings. Preparation of sample libraries is a critical step of the NGS workflow, and can be a time-consuming, labor-intensive, and costly process. Quality control of input samples, at intermediate steps of the preparation process, and of the final libraries before sequencing, can help save time and resources by identifying samples that are of poor quality or of insufficient concentration to yield successful sequence data.

The Agilent automated electrophoresis portfolio offers several instruments for QC analysis of nucleic acids, including the Agilent 2100 Bioanalyzer system, Fragment Analyzer systems, TapeStation systems, and Femto Pulse system.



Recommended steps for quality control during NGS library preparation.

Agilent 2100 Bioanalyzer System

The Agilent 2100 Bioanalyzer system is an easy-to-use benchtop platform with ready-to-run kits for a wide range of applications.

DNA size and quantity
Offering high resolution separation, sizing, and quantification of DNA fragments, smears, and NGS libraries down to pg/µL sensitivity.

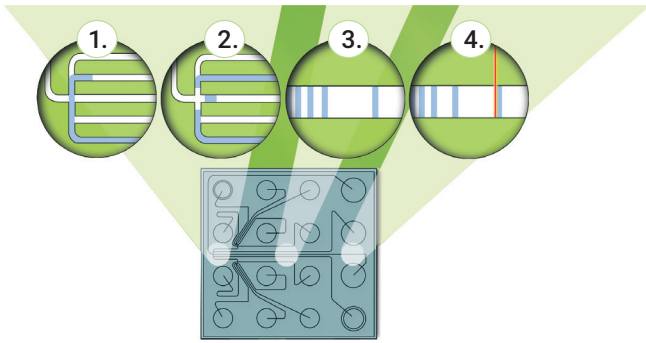
RNA quality check with RIN
Objective integrity analysis of total RNA is provided with the RIN (RNA integrity number). It is also possible to use the DV_{200} metric to assess quality of FFPE RNA samples before RNA sequencing studies.

Key benefits

- Minimal sample consumption with only 1 µL of sample required.
- High sensitivity down to pg/µL concentrations.
- Optimized applicaiton kits enable a quantitative range covering two orders of magnitude.
- High resolution, as good as 5 bp from fragments that are 300 bp and under.
- High-quality digital data for RNA, DNA fragment analysis and DNA library quality control.
- RIN algorithm for RNA sample integrity.
- Removable electrode cartridge for easy maintenance.
- 2100 Expert Security Pack and IQ/OQ services facilitate 21 CFR Part 11 compliance.

Microfluidic-based electrophoresis increases the quality and efficiency of your analysis

Microfluidic technology has many advantages over conventional methods, such as gel electrophoresis. The fully-automated sample analysis and data gathering process offers reliable, reproducible and user-independent results. Sample separation on a microchip allows high resolution with minimal sample consumption.



Principle of operation
1. The sample moves through the microchannels from the sample well.
2. The sample is injected into the separation channel.
3. Sample components are electrophoretically separated.
4. Components are detected by their fluorescence and translated into gel-like images (bands) and electropherograms (peaks).

Reagent kits for the 2100 Bioanalyzer system

Numerous NGS protocols recommend the Bioanalyzer for QC during library preparation. Agilent offers many kits for the Bioanalyzer system, to assess quality and quantity of DNA and RNA samples.

The **Agilent High Sensitivity DNA kit** provides sizing, quantification, and molarity assessment of DNA fragments, smears, and NGS libraries in the 50 to 7,000 bp size range, down to pg/µL sensitivity.

Agilent DNA kits enable DNA analysis up to 12,000 bp. Sizing and quantification are especially useful for sample quality control and the monitoring of critical steps in NGS workflows, including DNA fragmentation, target enrichment, and DNA library amplification.

The **Agilent RNA 6000 Nano kit** is a well-established standard for RNA sample quality control.

The **Agilent RNA 6000 Pico kit** allows detection of RNA degradation with sample concentrations down to 50 pg/µL of total RNA.

The **Agilent Small RNA kit** enables microRNA and small RNA analysis for assessing starting material quality in small RNA NGS library preparation.The kit ranges from 6 to 150 nt, allowing separation of small RNA species with high resolution. The percent microRNA is automatically calculated by region analysis.

Due to the omnipresence of RNases and the instability of RNA, integrity checks and sample quantification are essential steps before any RNA-dependent experiment.

Agilent 2100 Expert Software

The **Agilent 2100 Expert software** generates the unambiguous RNA integrity number (RIN), provides a quantification estimate, calculates ribosomal ratios of total RNA samples, and automatically detects ribosomal RNA contamination in mRNA. Specific software assays can be selected for both the RNA Nano and Pico kits to analyze eukaryotic, prokaryotic, plant, and mRNA samples.

The optional **Agilent 2100 Expert Security Pack add-on** enables 21 CFR Part 11 compliance of your Bioanalyzer system for regulated environments. It addresses requirements, such as electronic signatures, audit trails, and user authentication. Along with IQ and OQ support services and declarations of conformity for components offered for all assays and kits, your Bioanalyzer system will be compliant in no time.

Agilent Fragment Analyzer Systems

The Agilent Fragment Analyzer systems use automated parallel capillary electrophoresis to provide reliable quality control (QC) of nucleic acids for various applications, including next-generation sequencing (NGS).

The Fragment Analyzer systems break through analytic bottlenecks and streamline nucleic acid analysis workflows, providing researchers with the results they need. Automated parallel capillary electrophoresis can analyze multiple samples at once without researcher intervention. The systems can house two different gel matrices enabling unattended and consecutive analysis of multiple RNA and DNA reagent kits.

Key benefits

- Flexibility of interchangeable arrays, offering adjustable throughput to fit the changing needs of any lab.
- Ability to load up to three 96-well plates and process in any order.
- Minimal sample concentration requirements enable researchers to conserve precious samples for further analysis.

With three models, the Fragment Analyzer systems are easily adaptable to the workflow of any lab:

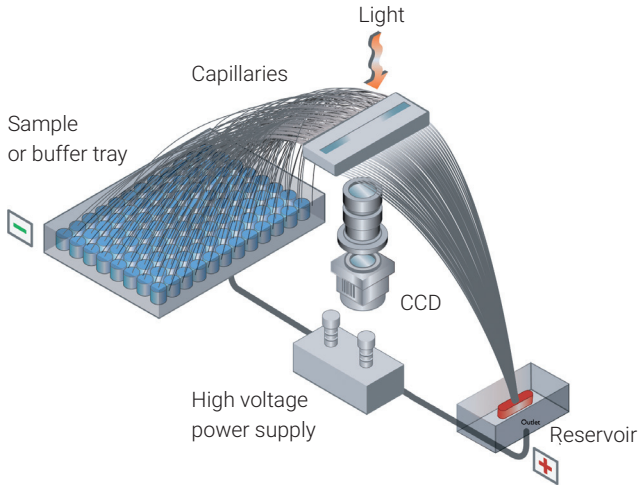
The **Agilent 5200 Fragment Analyzer** uses a 12-capillary array and is ideal for low-to-medium throughput labs.

The **Agilent 5300 Fragment Analyzer** offers higher throughput, with the ability to run a 48- or a 96-capillary array.

The **Agilent 5400 Fragment Analyzer** is an ultrahigh-throughput system, with a 96-capillary array. It is capable of full integration with most robotic systems using a tested Application Program Interface (API). The system can analyze thousands of samples per day.

Capillary electrophoresis

Capillary electrophoresis offers many advantages over conventional nucleic acid analysis methods, such as agarose gel electrophoresis. The capillary arrays are the basis of the Fragment Analyzer systems, as they can reliably separate both DNA and RNA samples, easily switching between applications. The arrays are available in three different lengths (short, ultrashort, and long) to allow the user to prioritize separation, resolution, or time, depending on workflow needs. A shorter capillary array offers a faster run time, while longer capillary arrays offer improved separation resolution.



Principles of capillary electrophoresis operation:

1. Individual capillaries are filled with gel.
2. Samples are voltage injected into the capillaries, and each sample moves through an individual capillary in a size-dependent manner.
3. As the fragments pass the detection window, a sensitive Charged Coupled Device (CCD) detector captures the size and concentration level of the sample.
4. The resulting series of images are merged together to produce a high-resolution electropherogram across the entire sizing range.

Further benefits of the Fragment Analyzer systems

- Separate multiple samples in parallel in as little as 15 minutes.
- Choose between three different capillary array lengths for the required blend of speed or resolution.
- Minimize instrument preparation time with no daily array handling requirements and room-temperature stable reagents.
- See clear results with separation resolution as good as 3 bp from fragments that are 300 bp and under.
- Improve lab efficiency by loading and programming samples while a separation is currently running.
- Easily adjust run priorities by rearranging the sample queue after runs have been loaded.
- Use quality metrics for RNA (RNA quality number, RQN) and genomic DNA (Genomic DNA quality number, GQN) to remove subjective quality assessments.
- Kits provide a wide concentration range covering two orders of magnitude.
- Achieve accurate molarity calculations with reliable smear analysis.

Reagent kits for the Fragment Analyzer systems

A broad range of kits are available for the Fragment Analyzer systems, allowing you to easily qualify and quantify both DNA and RNA samples. The instruments can separate diverse sample types, making them ideal for individualized workflows, including NGS library QC.

The **Agilent Small Fragment and NGS kits** facilitate the separation of DNA fragments and smears or NGS libraries,

with sizing from 50 to 1,500 bp and 100 to 6,000 bp, respectively. The Small Fragment and NGS Fragment kits cover a concentration range of 0.1 to 10 ng/µL for fragments and 5 to 100 ng/µL for smears. The HS Small Fragment and HS NGS Fragment kits have a smaller concentration range of 5 to 500 pg/µL for fragments and 50 to 5,000 pg/µL for smears. Each of the kits provide accurate quantification and sizing, making them ideal for NGS library preparation workflows.

The **Agilent Genomic DNA kits** were developed for the separation of genomic DNA (gDNA). Automated assessment of gDNA size and integrity is extremely beneficial for quality control of samples to be used in long-read and whole-genome sequencing, and analysis of degraded DNA. A broad sizing range allows accurate and precise sizing of samples up to 60 kb. Covering expansive concentration ranges, the HS Genomic DNA 50 kb kit is for samples ranging from 0.3 to 12 ng/µL, while the gDNA 50 kb kit extends from 25 to 250 ng/µL. gDNA samples can be easily analyzed with a quality metric, the GQN, which allows a user to define a threshold for what qualifies as good DNA for their purposes.

The **Agilent Large Fragment kits** are used for automated qualitative and quantitative analysis of large DNA fragments and smears up to 50 kb. Each of the kits has a different input concentration range, specific for fragments or smears. The Large Fragment kits are ideal for reliable quality control checkpoints for long-read sequencing. By comparing the sizing of sheared and unsheared large DNA samples, the kits provide a reliable evaluation of DNA fragmentation, an essential step in the preparation of large insert libraries.

The **Agilent RNA kits** can be used for analysis of both total RNA and mRNA, including IVT mRNA sizing. Ensuring quality RNA is crucial to many downstream applications, including NGS and gene expression studies. RNA analysis with the RNA kits provides each sample with a quality metric, known as the RNA quality number (RQN). Excellent resolution allows for distinction between small RNA and degraded RNA providing a reliable and accurate RQN score. Specific RNA analysis modes are available for eukaryotic, prokaryotic, plant, and mRNA samples. The RNA kits have a sizing range of 200 to 6,000 nt. The RNA kit (15 nt) covers a concentration range of 5 to 500 ng/µL, while the HS RNA kit is for less concentrated samples, with a range of 50 to 5,000 pg/µL.

The **Agilent Small RNA kits** focus on microRNA and small RNA quality control analysis, which is essential for downstream workflows such as small RNA NGS library preparation. The kits provide accurate and precise quantification and sizing of small RNA and microRNA, focusing on the narrow range from 15 to 200 nt. Concentrating on this small size range enables for high resolution separation and detailed analysis of both microRNA (10 to 40 nt) and small RNA (40 to 200 nt) regions. A region analysis function automatically calculates the percent microRNA and quantifies the microRNA and small RNA regions.

Agilent TapeStation Systems

Building on the success of the Agilent 2100 Bioanalyzer system, the Agilent TapeStation systems offer scalable throughput and rapid results, making them the ideal solution for quality control of biological samples in next-generation sequencing (NGS).

While the higher throughput **Agilent 4200 TapeStation system** can analyze DNA and RNA samples from a 96-well plate, the smaller footprint **Agilent 4150 TapeStation instrument** is the lower throughput alternative for analyzing up to 16 samples per run. Both systems offer walk away operation with fully automated sample processing.
No matter which system you choose, full assay compatibility is guaranteed as both instruments share the same ScreenTape consumables.

Advantages of ScreenTape technology

The TapeStation systems use credit card-sized ScreenTape devices that are available for DNA and RNA applications. Sample analysis has never been so easy – simply load the TapeStation instrument with ScreenTape devices, loading tips, and your samples in either 2 x 8 tube strips or a 96-well plate. After automated electrophoresis and imaging, quantification, sizing, and integrity data are available in as little as 1 to 2 minutes per sample. The ready-to-use ScreenTape technology enables ultimate flexibility for switching between applications. With sensitive detection and zero carryover, you get confidence in your results, ensuring your downstream workflow is a complete success.

The **Agilent TapeStation software** includes intuitive functionality for instrument control and data analysis. Automatically analyzed data is presented as a familiar gel image and there is an electropherogram for each sample. With a few mouse clicks, the software generates customized reports and exports data in different formats, ready to be imported into a LIMS system.

Key benefits

- Simplify your workflow with fully automated sample processing and ready-to-use ScreenTape technology.
- Up to 96 samples can be analyzed with constant cost-per-sample providing scalable throughput.
- Partially used ScreenTape devices can be reused at a later point of time.
- Easily switch between DNA and RNA ScreenTape assays for greatest flexibility.
- Results are obtained in as little as 1 to 2 minutes per sample.
- Achieve user-independent results with minimal manual intervention and excellent reproducibility for sizing, concentration and integrity assessments.
- Requires only 1 to 2 µL of DNA or RNA sample per run, even for high-sensitivity analysis.
- Reliable integrity standards for RNA (RNA integrity number equivalent, RIN^{e}) and genomic DNA (DNA integrity number, DIN).
- Availability of compliance services (IQ and OQ/PV) for TapeStation hardware and software.
- Each sample is analyzed in a separate lane with individual loading tips, eliminating carryover.
- Sample evaporation is avoided by covering the 96-well plate with pierceable foil.
- Complete assay compatibility guarantees seamless switching between the systems.

ScreenTape assays for the TapeStation systems

The **Agilent D1000 ScreenTape assay** facilitates the separation and analysis of DNA fragments, sheared DNA, and NGS libraries from 35 to 1,000 bp. Choose between the D1000 and the **Agilent High Sensitivity D1000 ScreenTape** assays depending on the sensitivity requirements of your

application. Both kits offer accurate and reliable sizing and quantification with a sample input of only 1 to 2 µL. The High Sensitivity D1000 ScreenTape assay has a sensitivity of 5 pg/µL for DNA fragments.

The **Agilent D5000 ScreenTape assay** can separate and analyze DNA fragments from 100 to 5,000 bp, ideal for larger sized NGS libraries. Depending on the sample concentration, choose between the D5000 ScreenTape and the **Agilent High Sensitivity D5000 ScreenTape assay**.

The **Agilent Genomic DNA ScreenTape assay** was developed for the separation and analysis of genomic DNA (gDNA) from 200 to greater than 60,000 bp. It provides accurate quantification and sizing data, as well as an automated numerical assessment of gDNA quality, based on the DNA integrity number (DIN). This assay is the ideal QC tool for NGS and array comparative genomic hybridization (aCGH) workflows. The DIN algorithm is included in the TapeStation analysis software and provides a numerical assessment of the DNA quality by assigning each sample a score from 1 to 10. A high DIN indicates highly intact gDNA, and a low DIN suggests a strongly degraded gDNA sample. The Genomic DNA ScreenTape assay allows sample quantification in the range of 10 to 100 ng/µL. The functional range for the DIN number is between 5 and 300 ng/µL.

The **Agilent Cell-free DNA ScreenTape assay** provides an easy way to analyze the quality of cfDNA, providing quantification and sizing information up to 800 bp, as well as detection of high-molecular weight DNA contamination. The %cfDNA value gives an objective measurement of cfDNA quality, based on a preset region in the electropherogram. Only 2 µL of precious sample is required to detect cfDNA with high sensitivity down to 20 pg/µL. Determining the quantity and quality of cfDNA is crucial for the success of downstream experiments, such as NGS, droplet digital PCR (ddPCR) or microarray analysis.

The **Agilent RNA ScreenTape assay** provides efficient and reliable RNA analysis for RNA characterization and quality assessment. The RNA integrity number equivalent (RIN^e) delivers an instant and objective evaluation of eukaryotic and prokaryotic total RNA degradation. When assessing RNA integrity, the RIN^e number is directly comparable to the widely accepted and frequently cited RNA integrity number (RIN) from the Agilent 2100 Bioanalyzer system. A high RIN^e indicates highly intact RNA, and a low RIN^e a strongly degraded RNA sample. The RNA ScreenTape assay also provides quantitative information and the ribosomal ratio of eukaryotic and prokaryotic RNA samples. In addition, the TapeStation software can provide the DV_{200} value for RNA samples isolated from FFPE tissue.

Choose between the RNA ScreenTape and the **Agilent High Sensitivity RNA ScreenTape assay** depending on the sensitivity requirements of your application. The High Sensitivity RNA ScreenTape assay can detect RNA samples down to 100 pg/µL.

Agilent provides eight different ScreenTape assays for various applications.

Agilent Femto Pulse System

The Agilent Femto Pulse system delivers unparalleled sensitivity and sizing of nucleic acids. Easily switch between DNA and RNA applications, with detection into the femtogram range. This system enables accurate and reliable sizing of gDNA smears and large fragments through 165 kb.

The Femto Pulse system is a powerful and effective pulsed-field capillary electrophoresis system. An automated pulsed-field power supply gives the system the ability to separate high-molecular weight DNA through 165,000 bp. An optimized optical platform allows the system to easily achieve 10 times higher sensitivity for nucleic acid smears and 100 times higher for nucleic acid fragments. The Femto Pulse system was designed for the qualitative and quantitative analysis of nucleic acid samples with low concentrations or high molecular weight. The system is therefore ideal for QC analysis of samples for downstream applications, including single-cell analysis, cfDNA, PCR-free libraries, RNA, genomic DNA, and long-read sequencing libraries.

Key benefits

- Quickly and accurately quantify, qualify, and size DNA fragments through 165 kb.
- Enhanced sensitivity allows for detection of DNA fragments down to 50 fg/µL.
- Easily replace overnight pulsed-field gel electrophoresis (PFGE) in approximately 1.5 hours without compromising separation resolution.
- Unparalleled sensitivity allows you to use less precious sample and achieve superior detection.
- Separate and quantify a single cell's worth of genomic DNA or total RNA.

Pulsed-field power and capillary electrophoresis

The Femto Pulse system utilizes a pulsed-field power supply, enabling pulsed-field capillary electrophoresis of samples through 165 kb in approximately 1.5 hours, eliminating overnight PFGE. Similar to the Fragment Analyzer systems, capillary electrophoresis is the foundation of the Femto Pulse system. The system can also operate on direct current for applications that do not require pulsing. It offers reliable separation of both DNA and RNA samples, and a low-maintenance capillary array makes it easy to switch between applications. The system is compatible with a 12-capillary array, designed for enhanced resolution. The capillary array and enhanced optical platform of the Femto Pulse system deliver unprecedented sensitivity, meaning that samples can be detected down to the femtogram concentration range.

Further benefits of the Femto Pulse system

- Large fragment resolution – Separate high-molecular weight DNA smears and fragments through 165 kb in approximately 1.5 hours.
- Femtogram level sensitivity – Achieve 10 times higher sensitivity for nucleic acid smears and up to 100 times higher for nucleic acid fragments.
- Powerful analysis software – One intuitive software program automates analysis of diverse sample types.
- Make sequencing workflows more efficient – Eliminate overnight pulsed-field gel electrophoresis from long-read sequencing library preparation and BAC analysis.

Reagent kits for the Femto Pulse system

A broad range of kits are available for the Femto Pulse system, allowing you to easily qualify and quantify both DNA and RNA samples. These instruments can separate diverse sample types, making them ideal for individualized workflows, including NGS library QC.

The **Agilent Genomic DNA 165 kb kit** was developed for easy and fast QC of high-molecular weight (HMW) gDNA and long-read sequencing libraries, eliminating traditional overnight PFGE protocols. Automated pulsed-field methods have been optimized for separation and sizing of HMW gDNA through 165 kb in as little as 70 minutes or 3 hours for enhanced resolution. A user-defined quality metric, the genomic quality number (GQN), enables unbiased assessment of sample integrity, depending on the needs of the downstream application.

The **Agilent Ultra Sensitivity NGS kit** is used for quality control of samples at extremely low concentrations throughout the NGS library preparation process. Reliable sizing of samples is achieved from 100 to 6,000 bp and quantification of DNA smears and fragments into the femtogram range. DNA smears can be detected down to 5 pg/µL, and accurately quantified through 250 pg/µL. Additionally, the DNA fragment detection range is expansive, covering 50 fg/µL to 5 pg/µL. The enhanced sensitivity of this kit allows conservation of precious samples, making it ideal for QC of low concentration NGS libraries, PCR-free libraries, and cfDNA.

The **Agilent Ultra Sensitivity RNA kit** can be used for assessment of both total RNA and mRNA from 200 to 6,000 nt at low concentrations. Two optimized methods allow for detection down to 2.5 pg/µL total RNA or 15 pg/µL mRNA, and quantification up to 250 pg/µL total RNA or 500 pg/µL mRNA. Powerful analysis software provides an objective quality score, the RNA quality number (RQN), for each sample and calculates the percent ribosomal contamination for mRNA. Accurate sizing and quantification of RNA samples can be achieved, necessary for quality control steps in NGS library preparation workflows.

Quality Metrics Overview

High-quality nucleic acids are necessary for successful library preparations and sequencing results. Nucleic acid quality control (QC) can determine which samples are not suitable for library preparation. Not all extraction methods are the same, resulting in nucleic acids with varying integrity. Nucleic acid samples such as formalin-fixed paraffin-embedded (FFPE), ancient samples, and RNA are easily degraded due to chemical fixation, time, temperature, enzyme digestion, and improper handling. If a sample is too far degraded, it will result in poor sequencing results, loss of coding areas of interest, and gaps in the full length of genomic DNA (gDNA). Knowing input nucleic acid quality helps provide guidance for changes needed to optimize a workflow, such as: input concentration, fragmentation conditions, the amount of library used in enrichment, and the number of PCR cycles to be used in amplification steps.

Quality metrics provide the user with a reliable assessment of the integrity of a sample. Users can establish quality metric standards in their workflows by examining the RNA integrity number (RIN) in RNA degradation studies, see page 49, or library preparation protocols with FFPE samples for DNA integrity number (DIN), see page 41. This saves time and money by reducing human error and variation between user assessments, while easily identifying unfit starting materials that would lead to poor results. The automated electrophoresis portfolio offers several instruments that can provide various quality metrics for different sample types.

Genomic DNA (gDNA)

Genomic DNA is easily sheared with everyday handling, mixing, and multiple freeze-thaw events. DNA from fresh, frozen, or FFPE tissue can be assessed with the following systems.

DNA integrity number (DIN) from the TapeStation systems
The DIN algorithm provides a numerical assessment of the DNA quality by assigning each sample a score from 1 to 10. A high DIN indicates highly intact gDNA, and a low DIN suggests a strongly degraded gDNA sample.

Genomic DNA quality number (GQN) from the Fragment Analyzer systems and Femto Pulse system
Agilent designed the GQN to allow for easy analysis of sheared DNA and gDNA quality. The user defines a size threshold they deem appropriate for their specific application. The GQN value is then calculated based on the fraction of the total measured concentration of the sample that lies above the specified size threshold. The GQN scores samples on a scale of 0 to 10, where 0 indicates that none of the sample exceeds the threshold and 10 indicates 100% of the sample lies above the threshold value.

Please see the Analysis of Genomic DNA section, page 28, and the Analysis of FFPE DNA section, page 39, for more information.

Cell-free DNA (cfDNA)

%cfDNA from the TapeStation systems
Cell-free DNA has become an important input material for NGS, creating the need for a reliable quality metric. High-molecular weight (HMW) DNA in the cfDNA sample can interfere with library yield and sequencing quality.

The TapeStation systems and Cell-free DNA ScreenTape assay feature a new quality metric, %cfDNA. This reflects the percentage of cfDNA subcomponents that are present in the preset region between 50 and 700 bp in relation to the total sample DNA. The %cfDNA metric allows the user to evaluate sample quality and identify whether a sample contains sufficient cfDNA for downstream processes. For more information on the analysis of cell-free DNA, see page 58.

RNA

RIN from the Bioanalyzer system, RIN^e from the TapeStation systems, and RQN from the Fragment Analyzer and Femto Pulse systems

RNA from fresh or frozen tissue can be assessed with the RNA integrity number (RIN), the RNA integrity number equivalent (RIN^e), or the RNA quality number (RQN). All three RNA quality metrics consider the entire electrophoretic separation of the RNA sample, including the ratio of the ribosomal bands and the presence or absence of degradation products. They are calculated using a scale from 1 to 10. A high RIN, RIN^e, or RQN indicates highly intact RNA, and a low number suggests a strongly degraded RNA sample. Several studies have been performed demonstrating the equivalences of the RIN to the RIN^e, see page 44, and the RIN to the RQN, see page 47, as highlighted in the Analysis of Total RNA section.

FFPE RNA

DV_{200} quality metric from the Bioanalyzer system, the Fragment Analyzer systems, the TapeStation systems, and the Femto Pulse system

FFPE RNA samples are difficult to use, as degradation due to fixation and storage conditions is often quite extensive. It is important to evaluate the quality of each FFPE RNA sample before proceeding with library preparation to eliminate highly degraded samples containing RNA fragments smaller than the optimal size range. Although RIN, RIN[e], and RQN values are reliable metrics for evaluating the quality of RNA isolated from fresh and frozen tissue or cell culture, it is not a definitive measure of RNA quality from FFPE samples. To solve this problem, the DV_{200} quality metric was developed. It calculates the percentage of RNA fragments greater than 200 nucleotides in size. The DV_{200} metric is then used to determine the minimal RNA input required for successful library preparation and reproducible results. Given the strong correlation between DV_{200} values and library yield, the DV_{200} metric is ideal for assessing FFPE RNA quality before library construction. Please see the Analysis of FFPE RNA section, page 55, for more information.

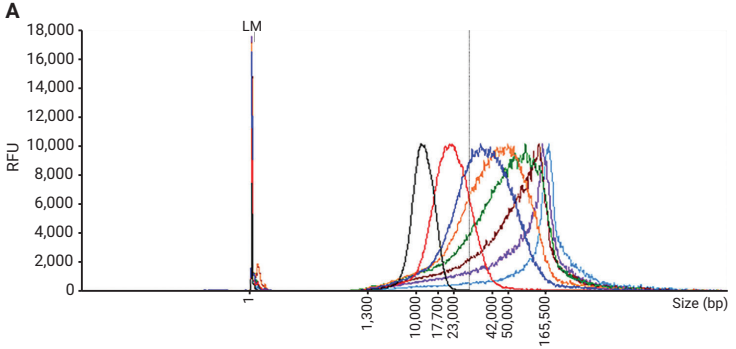
Overview of quality metrics				
	gDNA and FFPE DNA	cfDNA	RNA	FFPE RNA
Bioanalyzer system			RIN	DV_{200}
Fragment Analyzer systems	GQN		RQN	DV_{200}
TapeStation systems	DIN	%cfDNA	RIN[e]	DV_{200}
Femto Pulse system	GQN		RQN	DV_{200}

Overview of quality metrics for the Agilent Automated Electrophoresis portfolio.

Analysis of Genomic DNA

GQN quality metrics with the Fragment Analyzer and Femto Pulse systems

A



B

Average Smear Size (bp)	GQN Set at 30 kb
12,147	0
23,339	1.5
45,304	6.4
57,789	7.1
73,267	7.8
94,045	7.8
109,968	8.2
164,292	8.8

Instrument: **Fragment Analyzer and Femto Pulse systems**

Kit: **Genomic DNA 165 kb kit (Femto Pulse)**

Software assay: **FP-1002-22 gDNA 165Kb assay (Femto Pulse)**

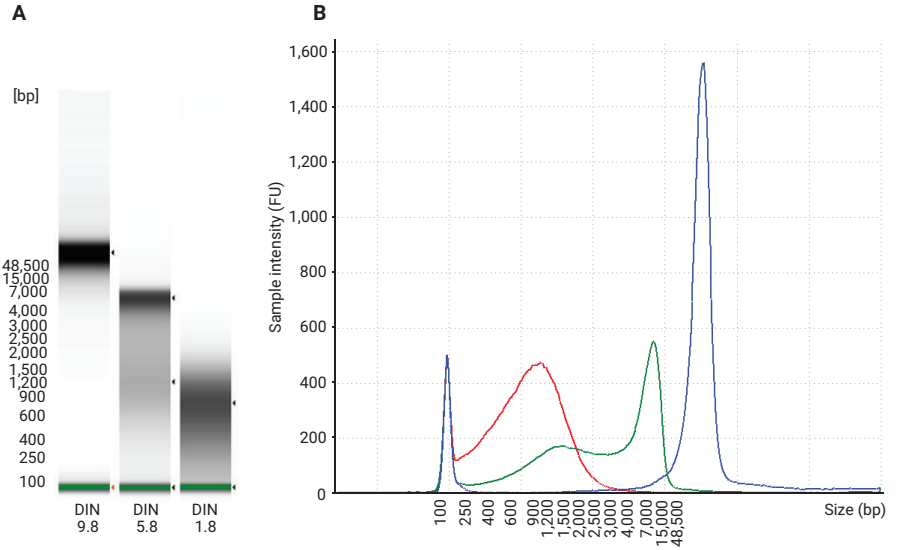
Abstract: Quality assessment of nucleic acids is critical to the success of many downstream applications, including next-generation sequencing (NGS). The Fragment Analyzer and Femto Pulse systems provide quick and easy assessment of genomic DNA (gDNA) quality and integrity with the genomic DNA quality number (GQN).

The GQN is commonly used for evaluating the input gDNA material for NGS library preparation. To prepare a successful library, the sample must be of the correct size and of sufficient quality for sequencing. The GQN threshold can be set by the user to reflect the size threshold necessary for their particular requirements. The GQN is given on a scale of 0 to 10, with a higher score indicating that more of the sample exceeds the user-defined threshold. In this example, the Femto Pulse system was used to report the average smear size (A) and GQN set at 30 kb (B) of several gDNA samples that had been sheared to various sizes. For NGS, quality metrics can aid decisions about library preparation, including which samples to use for input material, the number of PCR cycles, and the amount of library to use for enrichment.

Application note: 5994-0521EN

Analysis of Genomic DNA

DIN quality metrics with the TapeStation systems



Instrument: **TapeStation systems**

Assay: **Genomic DNA ScreenTape assay**

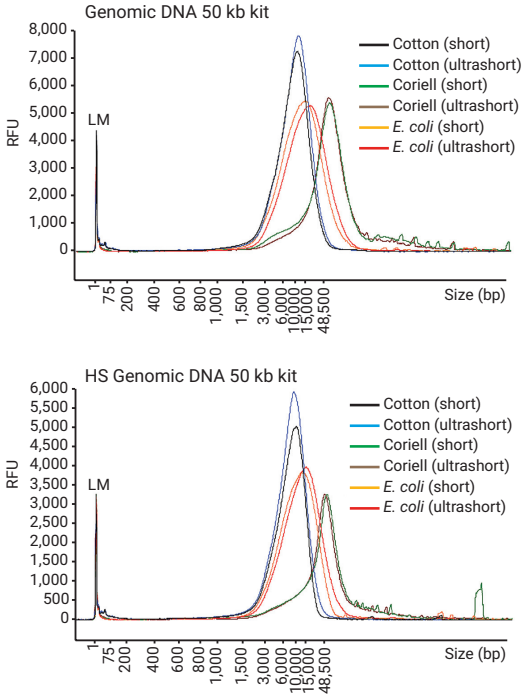
Abstract: When used with the Genomic DNA ScreenTape assay, the 4200 TapeStation system can separate and analyze genomic DNA (gDNA) from 200 to 60,000 bp. It provides an automated numerical assessment of gDNA quality, the DNA integrity number (DIN). The DIN is calculated using a scale from 1 to 10. A high DIN indicates highly intact gDNA, and a low DIN suggests a strongly degraded gDNA sample. The user-independent DIN is the ideal QC tool for next-generation sequencing (NGS) and array comparative genomic hybridization (aCGH) workflows.

Three different mouse gDNA samples with different DNA integrity were analyzed using the 4200 TapeStation system. The TapeStation analysis software displays the results as an electropherogram, a gel image, and data table. The DIN value is automatically determined, and directly displayed under the individual lane of the gel image (A). The corresponding samples are shown in the electropherogram overlay (B).

Technical overview: 5991-6629EN

Analysis of Genomic DNA

Consistent sizing of gDNA



Instrument: **Fragment Analyzer systems**

Kit: **Genomic DNA 50 kb kit, HS Genomic DNA 50 kb kit**

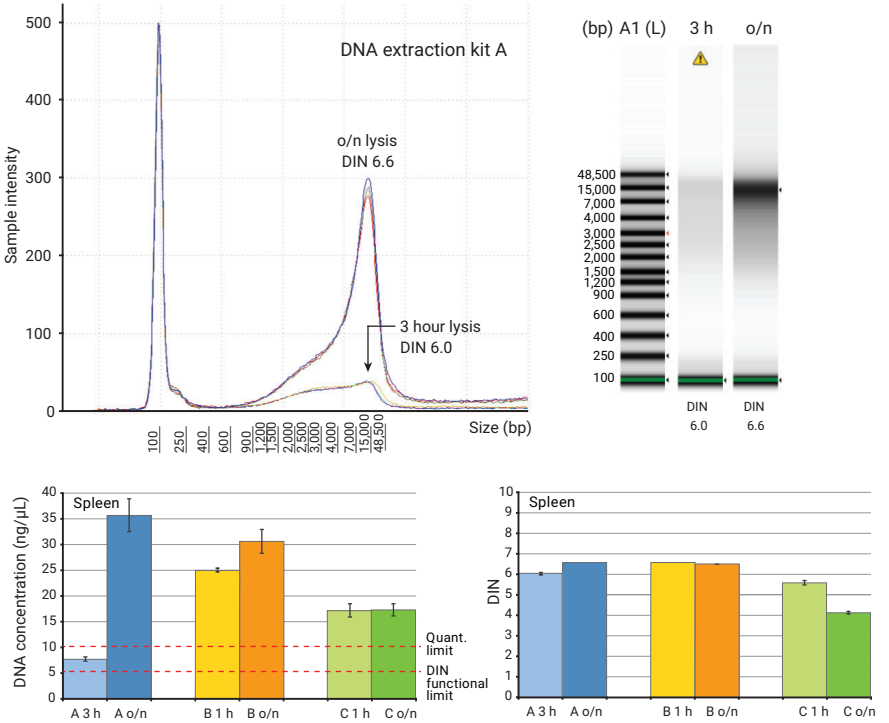
Software assay: **DNF-467-33-Genomic DNA 50Kb assay, DNF-467-22-Genomic DNA 50Kb assay, DNF-468-33-HS Genomic DNA 50Kb assay, DNF-468-22-HS Genomic DNA 50Kb assay**

Abstract: The quality and concentration of genomic DNA (gDNA) starting material is crucial for successful downstream long-read and whole-genome sequencing. Quality analysis for gDNA with varying ranges of concentrations can be performed on the Fragment Analyzer systems with the Genomic DNA 50 kb kit and the HS Genomic DNA 50 kb kit. The Genomic DNA 50 kb kit offers a concentration range of 25 to 250 ng/µL input gDNA, while the HS Genomic DNA 50 kb kit has a lower concentration range of 0.3 to 12 ng/µL input gDNA for low concentrated samples. Genomic DNA from cotton, *E. coli,* and human (Coriell) were compared on both kits with the FA 12-Capillary Array Short, 33 cm (short array), and FA 12-Capillary Array Ultrashort, 22 cm (ultrashort array). On both kits, the short and ultrashort arrays demonstrated consistent sizing for the three sample types. The ultrashort array offers the convenience of shortened run times while providing comparable gDNA sizing, concentration, and genomic quality number (GQN) compared to the short array with both kits.

Application note: 5994-0511EN

Analysis of Genomic DNA

Optimization of gDNA extraction from FFPE tissue



Instrument: **TapeStation systems**

Assay: **Genomic DNA ScreenTape assay**

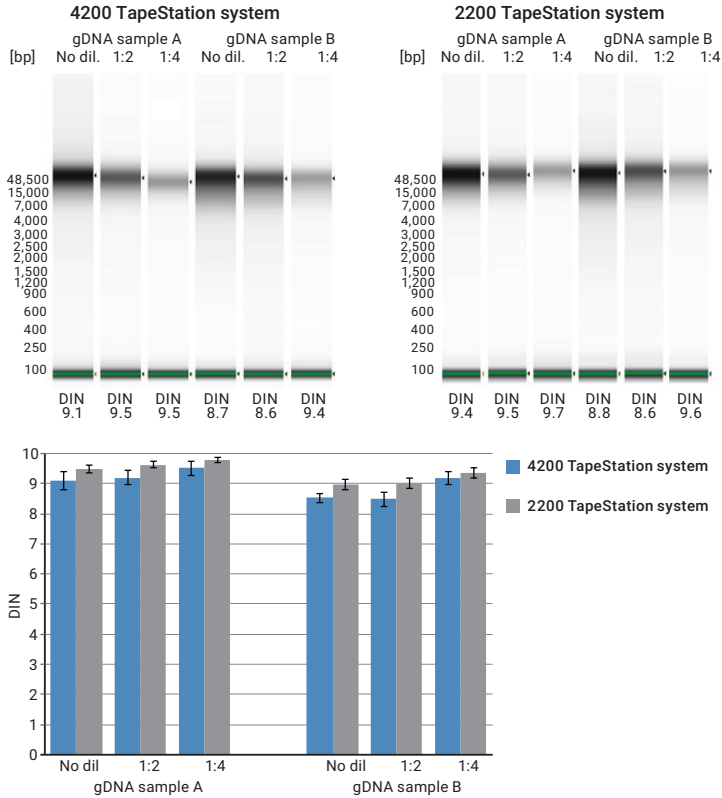
Abstract: One of the most widely used methods for tissue preservation and archiving is the preparation of formalin-fixed paraffin-embedded (FFPE) samples. These FFPE tissue archives represent a valuable source for retrospective studies of gene expression and mutation analysis. However, DNA extraction from FFPE samples has proven challenging. To compare the effect of DNA extraction methods and the influence of tissue type on the quantity and quality of the extracted DNA, different mouse tissues isolated by three commercially available DNA extraction kits (vendors A, B and C) were investigated. The electropherogram and the gel image shown in the figure above summarize the results obtained for the DNA extracted from mouse spleen FFPE tissue using one extraction kit.

The Genomic DNA ScreenTape assay and the DNA integrity number (DIN) is a valuable and reliable tool for quality control of DNA extraction, with quantification determination and automated sample integrity assessment. The DIN is presented automatically, and, therefore, does not require a subjective integrity estimation or approximation based on user experience.

Application note: 5991-5246EN

Analysis of Genomic DNA

DIN comparison across different TapeStation systems



Instrument: **TapeStation systems**

Assay: **Genomic DNA ScreenTape assay**

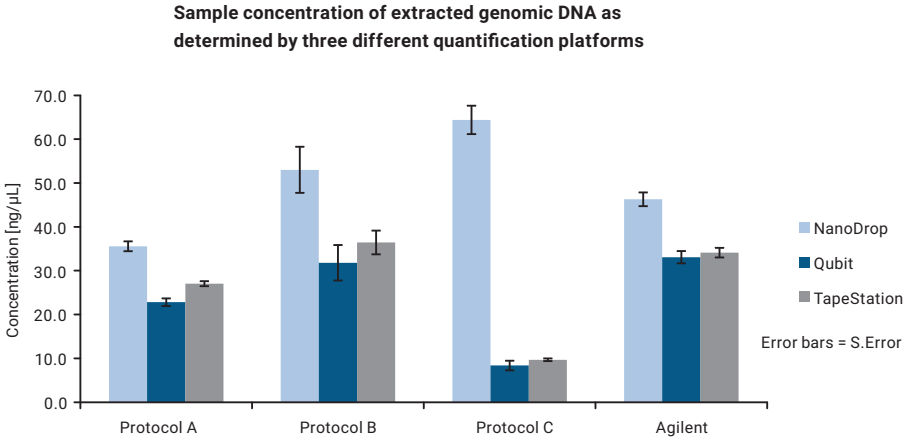
Abstract: To evaluate the difference of the DNA integrity number (DIN) between the 2200 and the 4200 TapeStation systems, two commercially available gDNA samples were analyzed at three different concentrations using the Genomic DNA ScreenTape assay. The DNA integrity number (DIN) is automatically determined and directly displayed below the individual lane of the gel image. A DIN is calculated on a scale from 1 to 10. A high DIN indicates highly intact gDNA, whereas a low DIN corresponds to a strongly degraded gDNA. Both gDNA samples were highly intact, as indicated by the determined DIN, which is displayed below the gel image.

The bar chart illustrates that the DNA integrity analysis with the 4200 TapeStation system is highly comparable to that of the 2200 TapeStation system. The precision for the DNA integrity analysis for both TapeStation systems is 4% for all tested samples and concentrations.

Application note: 5991-6892EN

Analysis of Genomic DNA

Quantification of isolated gDNA samples



Instrument: **TapeStation systems**

Assay: **Genomic DNA ScreenTape assay**

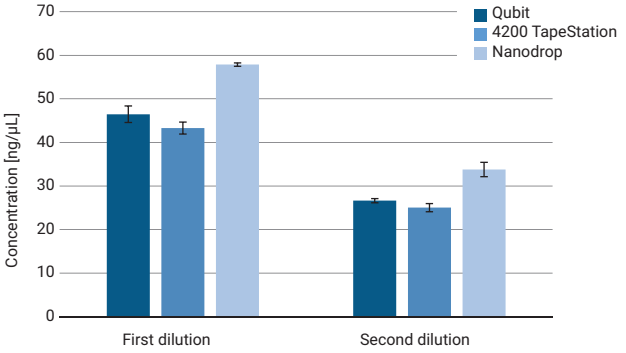
Abstract: When used with the 4200 TapeStation system, the Genomic DNA ScreenTape assay enables automatic analysis of up to 96 genomic DNA samples. With the DNA integrity number (DIN), the assay provides automatic determination of sample integrity and sample quantification. In the above example, genomic DNA from HEK293 cells was isolated with extraction kits from different suppliers. Samples were analyzed in triplicates and the quantification data was collected for each kit. The samples were also quantified using the Qubit dsDNA broad range assay and NanoDrop spectrophotometer to compare results. The mean values obtained from the Qubit, NanoDrop, and TapeStation system with Genomic DNA ScreenTape assay were plotted and compared.

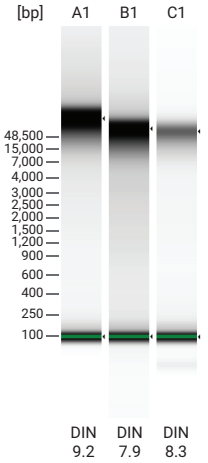
The data shows that the ScreenTape and Qubit analysis provide very similar results with a minimum average difference of 1% and a maximum average difference of 5%. The coefficient of variation (CV) is similar across all three platforms for each kit, illustrating differences between each triplicate extracted sample rather than any performance variances in the platforms.

Application note: 5991-1797EN

Analysis of Genomic DNA

Quantity and integrity analysis of gDNA for NGS





Instrument: **TapeStation systems**

Assay: **Genomic DNA ScreenTape assay**

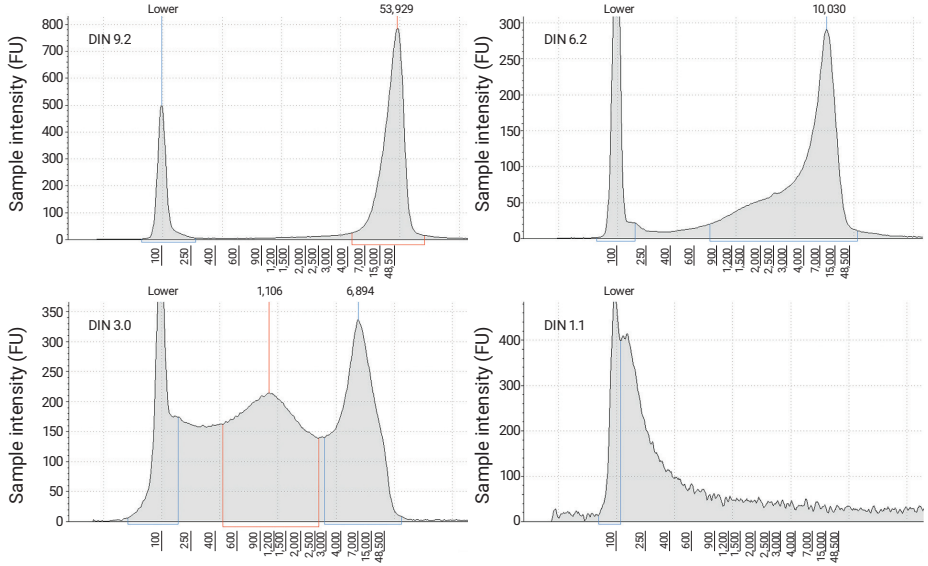
Abstract: The Agilent SureSelect QXT WGS protocol requires high-quality DNA samples for optimal performance and precise quantification of the gDNA starting material. Serial quantification was carried out using the Qubit instrument and the dsDNA BR assay in accordance with the protocol. The same samples were analyzed on the 4200 TapeStation system with the Genomic DNA ScreenTape assay and the NanoDrop with six replicates on each instrument. Data from the 4200 TapeStation system, Qubit, and NanoDrop are presented in the above figure, showing the applicability of the Genomic DNA ScreenTape assay in quantitating genomic DNA starting material. The quantification results of the TapeStation system correlate with the Qubit instrument. The measurement of genomic DNA with UV spectroscopy tends to overestimate the quantity due to other buffer components that may absorb in the UV spectrum.

In addition, the Genomic DNA ScreenTape assay provides an objective assessment of sample integrity within the same QC step. Sample integrity is automatically determined by the DNA integrity number (DIN) calculation provided by the TapeStation analysis software.

Application note: 5991-8191EN

Analysis of Genomic DNA

Integrity analysis of gDNA from bacterial sources



Instrument: **TapeStation systems**

Assay: **Genomic DNA ScreenTape assay**

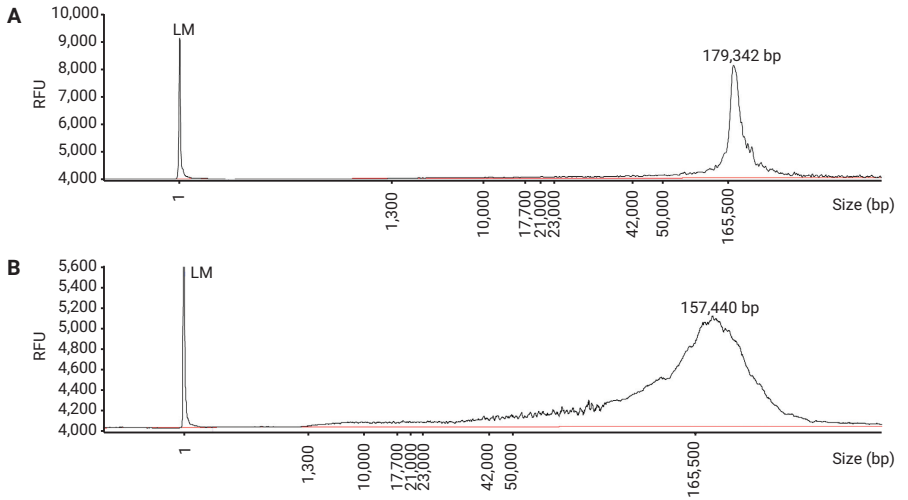
Abstract: The DNA integrity number (DIN) was developed to facilitate the quality control of isolated genomic DNA (gDNA) samples. The DIN software algorithm can classify total DNA based on a numbering system from 1 to 10, with 1 being the most degraded and 10 being the most intact. The DIN aids with interpretation of electropherograms, allows comparison of samples, and ensures the repeatability of experiments for gDNA moving into, for example, next-generation sequencing library construction.

These electropherograms illustrate the variability of the analyzed genomic DNA samples isolated from bacteria. DIN numbers range from 1.1 to 9.2. In earlier studies, samples with a DIN of > 7 were determined acceptable to progress into the next step of library construction. In this example, three out of four samples did not pass the QC criteria for the downstream application.

Application note: 5991-5442EN

Analysis of Genomic DNA

gDNA sizing and quality control



Instrument: **Femto Pulse system**

Kit: **Genomic DNA 165 kb kit**

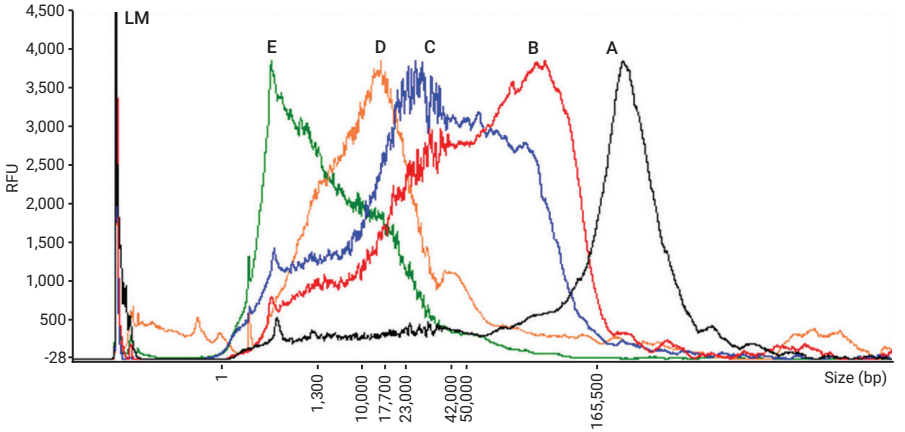
Software assay: **FP-1002-22-gDNA 165Kb assay, FP-1002E22 – Extended gDNA 165Kb assay**

Abstract: Large-insert library preparation relies upon multiple quality control steps. Typically, overnight pulsed-field gel electrophoresis (PFGE) separations are used to assess gDNA over 50 kb in size. The Agilent Femto Pulse system is the only instrument on the market capable of replacing PFGE with fast, automated assessment of high-molecular weight (HMW) gDNA, saving time and money in the preparation of large-insert libraries. The Genomic DNA 165 kb kit offers two pulsed-field capillary electrophoresis separation methods. The gDNA 165 kb method is a 70 minute method recommended for gDNA under 80 kb. The extended Genomic DNA 165 kb method provides enhanced separation and sizing for larger samples in 3.5 hours. Large gDNA separated with the fast method displayed a sharp, compact peak around 165 kb (A). The same sample analyzed with the extended method resulted in a broader smear representing the entire sizing range of the sample (B). This application note shows comparable sizing of four gDNA samples separated on the Femto Pulse and traditional PFGE. It also demonstrates consistent sizing of samples throughout a dilution series, and discusses the application of genomic quality number (GQN) provided by the Femto Pulse system.

Application note: 5994-0516EN

Analysis of Genomic DNA

Comparison of gDNA extractions



Extraction Method	Average Size* (bp)	% CV of Size	Average GQN*$_{50\ kb}$
A	163,670	1.9%	8.2
B	103,111	1.2%	7.4
C	76,778	2.8%	6.1
D	62,165	5.1%	3.0
E	27,388	4.7%	1.1

* n=3

Instrument: **Femto Pulse system**

Kit: **Genomic DNA 165 kb kit**

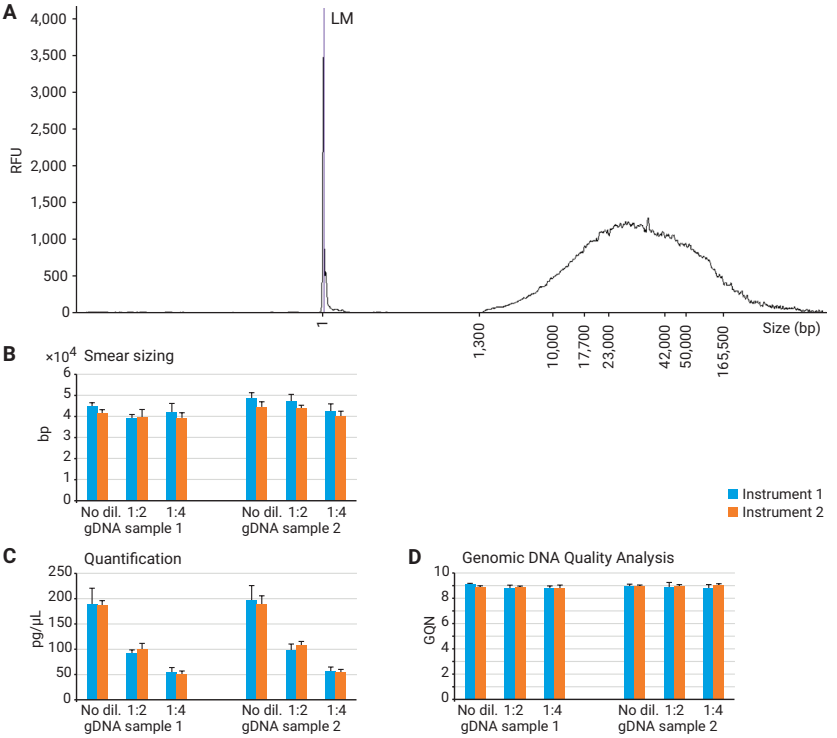
Software assay: **FP-1002-22-gDNA 165Kb assay, FP-1002E22 – Extended gDNA 165Kb assay**

Abstract: Various methods are available for isolation of gDNA from eukaryotic and prokaryotic sources, which are tailored towards the specific downstream applications. Consequently, the size and quality of the isolated gDNA is affected by the different extraction methods, driving the need for reliable quality assessment of gDNA samples. The Genomic DNA 165 kb kit was designed for separation of HMW gDNA on the Femto Pulse system, providing accurate and reproducible sizing and quality assessment. Sizing and quality of gDNA was compared between five different extraction methods. The yeast gDNA size distribution and integrity varied depending on the extraction method used. Method A, a phenol-chloroform extraction, and method B, another liquid extraction method, resulted in the largest intact gDNA smear. Method C, a spin column extraction method, and method D, a membrane extraction method, displayed similar sizing in the middle of the five different extraction methods. Method E, another spin column extraction method resulted in the smallest size. The genomic quality number, GQN, decreased with the decreasing size of the gDNA samples.

Application note: 5994-0754EN

Analysis of Genomic DNA

Assessment of gDNA for biobanking



Instrument: **Femto Pulse system**

Kit: **Genomic DNA 165 kb kit**

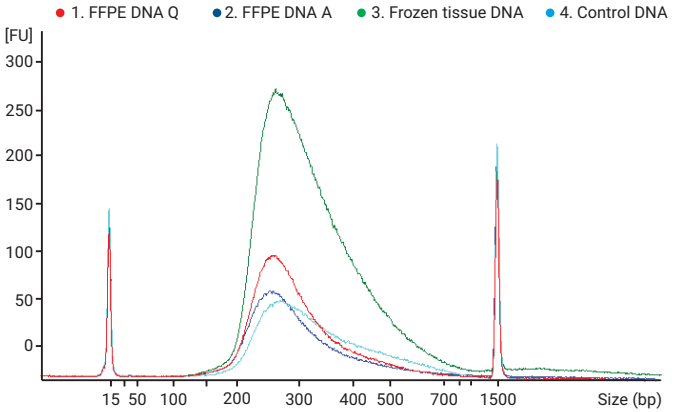
Software assay: **FP-1002-22-gDNA 165Kb assay**

Abstract: Characterization of input DNA integrity is important for NGS workflows. Growing interest in long-read and whole-genome sequencing technologies, comparative genomic hybridization, and mapping technologies using linked reads, has made knowledge of gDNA quality, fragmentation, sizing, and concentration a necessity for successful outcomes. Several factors affect sample quality such as: sample storage, extraction methods, repeated freeze-thawing, denaturation, and repeated mixing. Human gDNA samples 1 and 2 were separated on two different Femto Pulse systems with the Genomic DNA 165 kb kit to demonstrate reliable sizing, quantification, and quality analysis. The genomic quality number was set at 10 kb ($GQN_{10,000}$) between instruments (B, C, D). Sizing and GQN remained consistent over several concentrations (B, D). An electropherogram of sample 1 displays the size distribution of the gDNA sample (A). Quality metrics, such as the GQN, allow users to assess the starting quality of the gDNA sample.

Application note: 5994-0520EN

Analysis of FFPE DNA

Quality control of FFPE DNA samples



Sample	Average Size [bp]	Peak Height [bp]	Concentration [ng/µL]
FFPE DNA Q	307	264	34.8
FFPE DNA A	309	254	22.6
Frozen tissue DNA	331	264	89.5
Control DNA	348	271	23.2

Average size, peak height, and quantification of precaptured amplified libraries.

Instrument: **2100 Bioanalyzer system**

Kit: **DNA 1000 kit, High Sensitivity DNA kit**

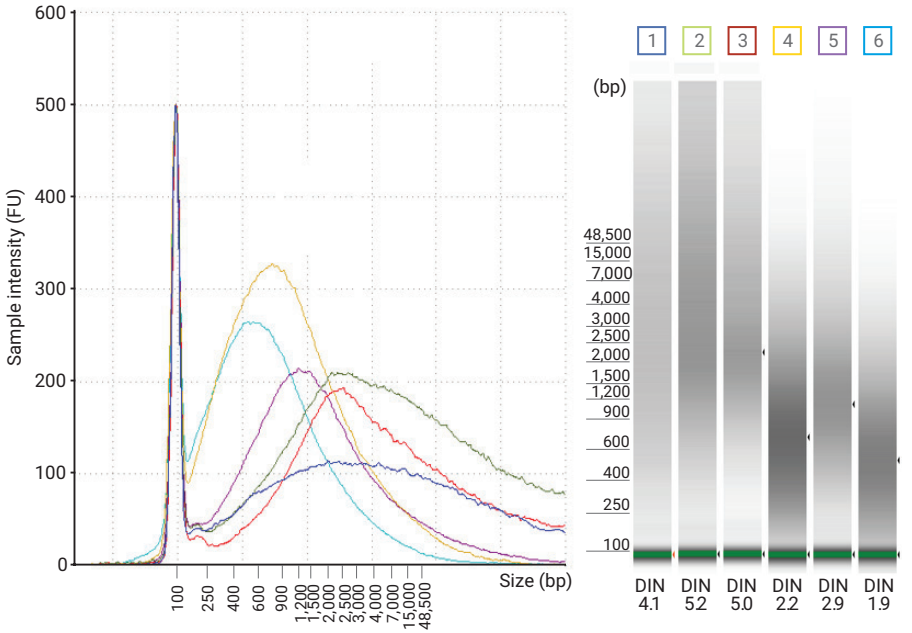
Abstract: The Bioanalyzer system was used for quality control of DNA samples from formalin-fixed paraffin-embedded (FFPE) and fresh frozen tissues before and during the SureSelect Target Enrichment workflow. The figure shows the electropherogram overlay of precapture amplified libraries after five PCR cycles run on the Bioanalyzer system with the DNA 1000 kit. Similar profiles were observed for all DNA samples. No amplification artifacts or primer dimers were seen. FFPE DNA samples gave comparable results to DNA from fresh frozen tissue and control cell line DNA, appropriate for downstream sequencing on the Illumina platform.

Reliable DNA electrophoresis with the Bioanalyzer system provided smear profiles and details for library characteristics, such as peak heights, average size, molarity and concentration.

Application note: 5991-0483EN

Analysis of FFPE DNA

Analysis of DNA from FFPE tissue



Instrument: **TapeStation systems**

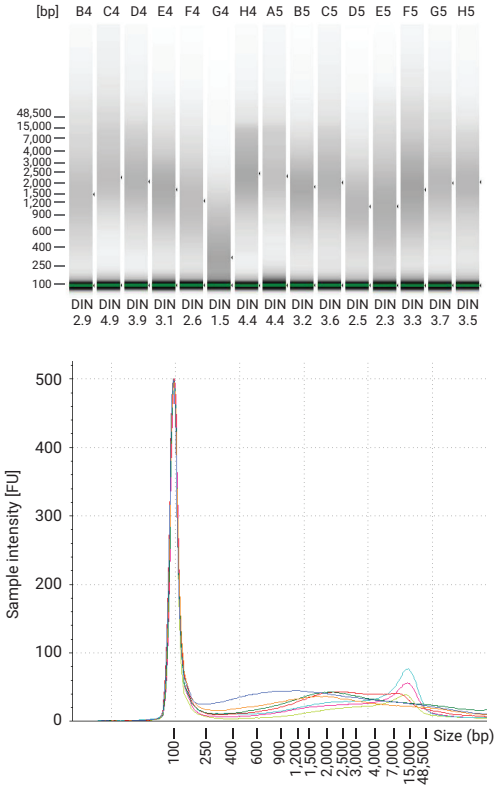
Assay: **Genomic DNA ScreenTape assay**

Abstract: Sequencing genomic DNA (gDNA) from FFPE archived tissue can be challenging, as the obtained material is often variable in quality. By establishing a customer specific threshold, the number (DIN) obtained by the Genomic DNA ScreenTape assay had saved sequencing and sample preparation overhead. The above data shows an overlay of electropherograms of different samples and the gel image with the determined DIN. Representative samples of sufficient DIN > 3 (samples 1–3) and insufficient DIN < 3 (samples 4–6) were selected for this figure. Out of a total of 751 FFPE samples, a subset of 197 were tested for a correlation of various NGS parameters against the DIN. A correlation was identified between DIN and the key parameters of on-target rate and coverage at 10x. The DIN correlates with key sequencing quality metrics and, therefore, presents an integrity threshold for the processing of FFPE samples.

Application note: 5991-5360EN

Analysis of FFPE DNA

Quality control of DNA from FFPE tumor samples



Instrument: **TapeStation systems**

Assay: **Genomic DNA ScreenTape assay**

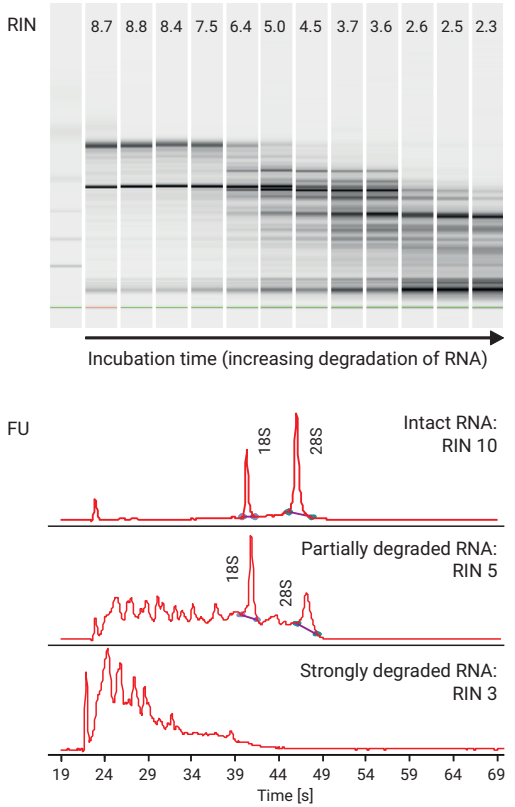
Abstract: To determine if samples were suitable for NGS library preparation, a quality control (QC) assessment was performed at the beginning for a batch of 88 genomic DNA (gDNA) samples from FFPE tumor tissue by the German Cancer Research Center (DKFZ) High Throughput Sequencing Unit. This initial QC includes quantification and analysis with a 4200 TapeStation system and the Genomic DNA assay to determine DNA quality, based on the DNA integrity number (DIN).

Both figures show a representative subset of samples analyzed on the 4200 TapeStation with the Genomic DNA ScreenTape assay. gDNA samples extracted from FPPE material often have low DNA integrity, but can still be sufficiently intact for whole-exome sequencing library preparation protocols and successful sequencing.

Application note: 5991-7615EN

Analysis of Total RNA

RIN quality metric with the Bioanalyzer system



Instrument: **2100 Bioanalyzer system**

Kit: **RNA 6000 Nano kit**

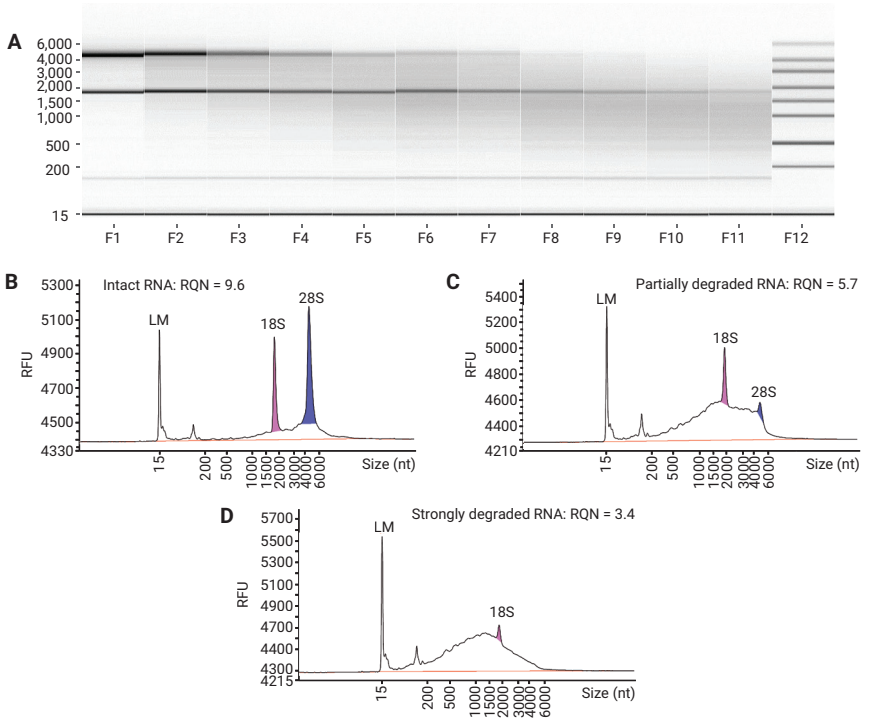
Software assay: **Eukaryote Total RNA Nano assay**

Abstract: The RNA integrity number (RIN) is calculated by a dedicated software algorithm to assess the quality of RNA preparations. The RIN tool is a major step in the standardization of user-independent RNA evaluation and delivers more meaningful information than simple ratio calculations for ribosomal RNA peaks. It is not influenced by instrument, sample integration, and most importantly, concentration variability. It therefore facilitates comparison of samples and avoids cost-intensive experiments with low quality RNA preparations. The RIN algorithm is based on a large collection of RNA data of various tissues and qualities. Anomalies like genomic DNA contaminations are indicated with weighted error messages (critical/noncritical) to achieve maximum reliability.

Application note: 5989-1165EN

Analysis of Total RNA

RNA quality control with the Fragment Analyzer systems



Instrument: **Fragment Analyzer systems**

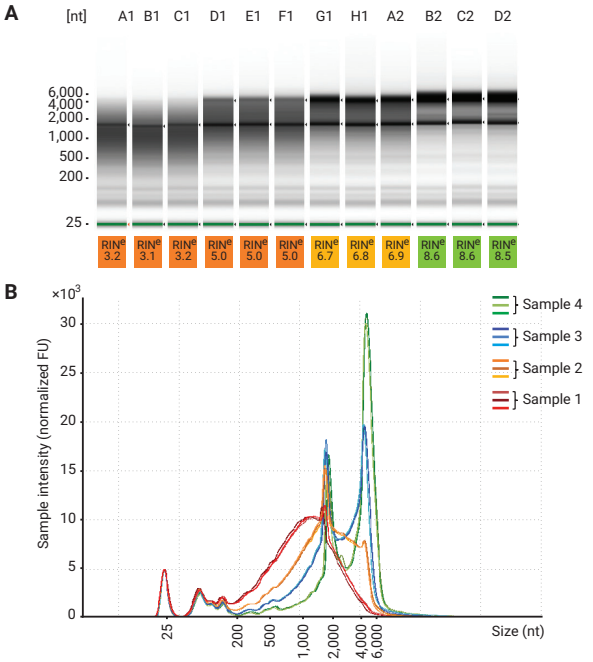
Kit: **HS RNA kit**

Abstract: The RNA quality number (RQN) is a user-independent quality metric for easy evaluation of total RNA quality. Total RNA quality is a constant concern because of how easily RNA degrades due to heat, RNase exposure, and improper handling. The 5200 Fragment Analyzer system and RQN metric were used to analyze universal mouse reference total RNA degradation over time. The digital gel image shows how the 18S ribosomal peak (pink color) becomes smaller, while the 28S ribosomal peak (blue color) completely disappears with heat degradation (A). Electropherograms allow for the total RNA profiles to be compared at different time points of heat degradation. High quality total RNA with no heat degradation has an RQN of 9.6 (B). Partially degraded total RNA has an RQN of 5.7 after 10 minutes at 70°C (C). Strongly degraded total RNA has an RQN of 3.4 after 16 minutes at 70°C (D). The RQN was consistent throughout the dilution series and between multiple 5200 Fragment Analyzer systems.

Application note: 5994-0519EN

Analysis of Total RNA

RIN^e quality metric with the TapeStation systems



Instrument: **TapeStation systems**

Assay: **RNA ScreenTape assay, High Sensitivity RNA ScreenTape assay**

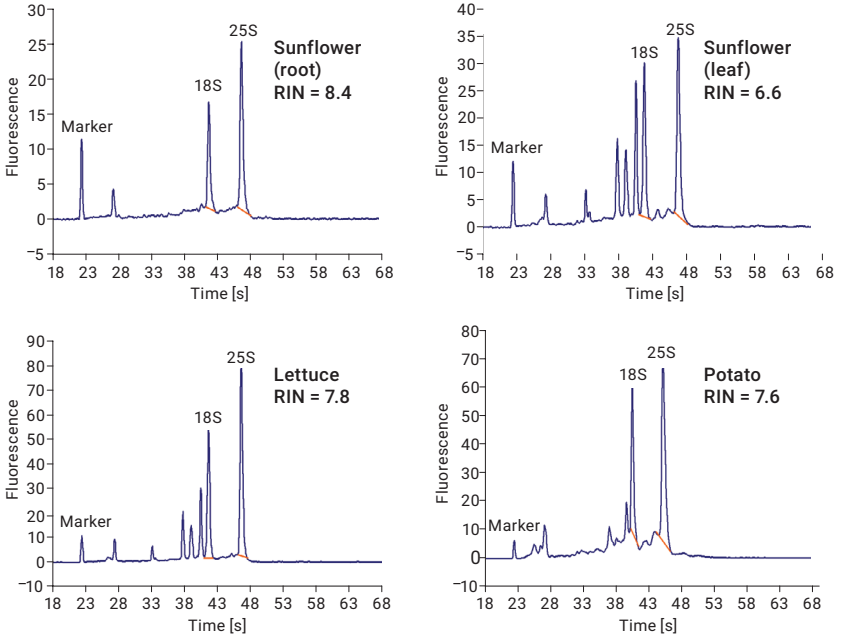
Abstract: RNA serves as input material within various gene expression analysis techniques like RNA-Seq, microarray, and RT-qPCR. RNA is very sensitive to degradation and its integrity critically affects the success of the downstream applications. Quality control of RNA input material is crucial for ensuring high-quality results. The 4150 TapeStation system can be used with the RNA ScreenTape assay to separate and analyze RNA, providing an automated numerical assessment of RNA quality, the RNA integrity number equivalent (RIN^e). The RIN^e is calculated using a scale from 1 to 10. A high RIN^e indicates highly intact RNA, and a low RIN^e suggests a strongly degraded RNA sample. The RIN^e was demonstrated to be equivalent to RIN from the 2100 Bioanalyzer system. The user-independent RIN^e is the ideal QC tool for next-generation sequencing (NGS) workflows.

Four rat kidney RNA samples with different degradation stages were analyzed using the 4150 TapeStation system. The 4150 TapeStation software displays the results as an electropherogram, a gel image, and data table. The RIN^e value is automatically determined, and directly displayed under the individual lane of the gel image (A). (B) shows the corresponding electropherogram overlay. Comparison of the RIN^e from the 4150 and 4200 TapeStation systems was evaluated and found to be equivalent.

Technical overview: 5994-1038EN

Analysis of Total RNA

Assessing integrity of plant RNA



Instrument: **2100 Bioanalyzer system**

Kit: **RNA 6000 Nano kit**

Software assay: **Plant RNA Nano assay**

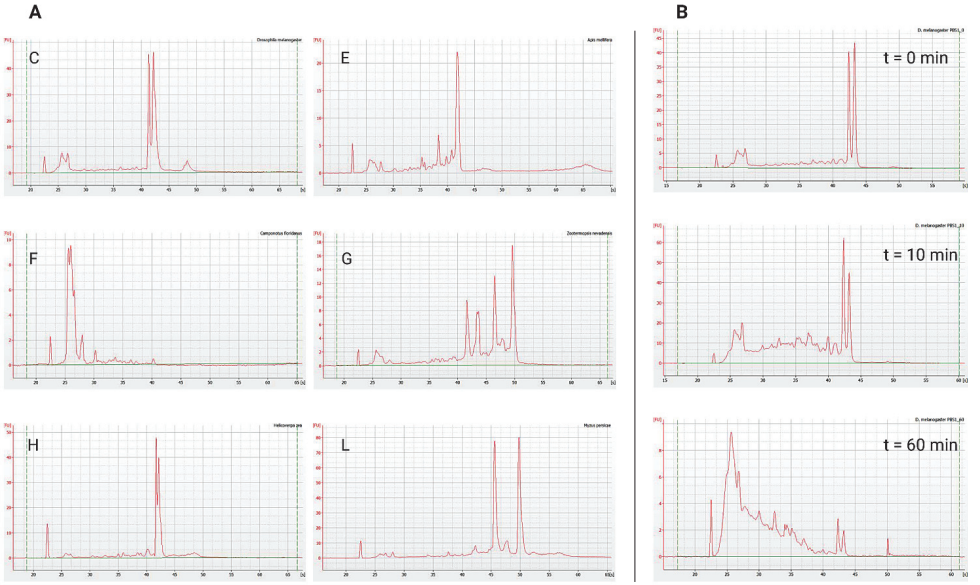
Abstract: High-quality RNA, free of genomic DNA, is a critical determinant for the success of many downstream techniques used in functional genomics, such as RT-PCR and microarray-based experiments. The dedicated plant RNA assay included in the 2100 Expert software (version B.02.07 or higher) allows rapid assessment of plant RNA integrity from multiple plant sources and differing degradation states with excellent precision. The figure shows the electrophoretic separation of different plant total RNA samples using the RNA 6000 Nano kit with the plant RNA assay. In all samples, the abundant 25S and 18S ribosomal RNA peaks are well resolved and automatically identified by the software. Compared to the RNA profiles of the root samples, the leaf and lettuce extracts exhibit additional fast migrating peaks corresponding to smaller chloroplast ribosomal RNAs. This shows that total RNA profiles can vary depending on species and tissue types.

The plant RNA assay and the RIN algorithm provide a convenient, user-independent assessment of total plant RNA integrity.

Application note: 5990-8850EN

Analysis of Total RNA

Assessing integrity of insect RNA



Instrument: **2100 Bioanalyzer system**

Kit: **RNA 6000 Nano kit, RNA 6000 Pico kit**

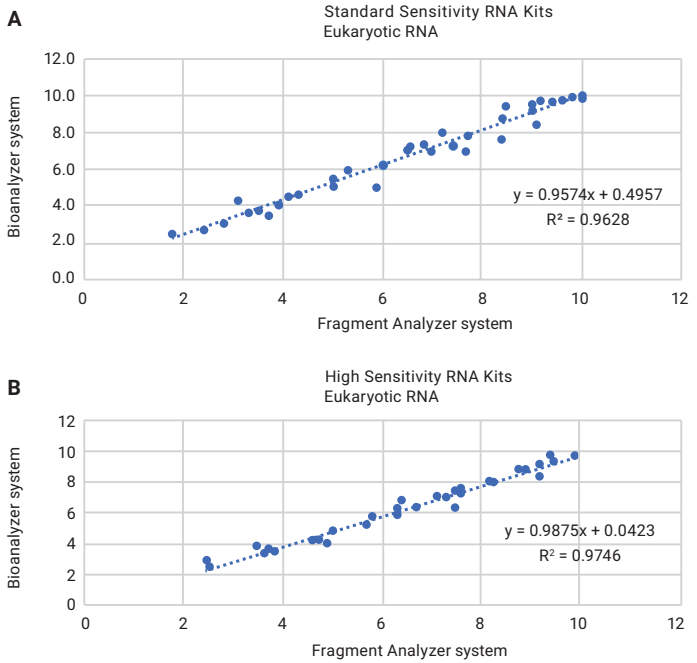
Software assay: **Eukaryote Total RNA Nano assay, Eukaryote Total RNA Pico assay**

Abstract: This application note describes the complexity of insect RNA and the challenges associated with integrity determination using the 2100 Bioanalyzer system. Here, various extracts of insect species were analyzed for total RNA quality using the 2100 Bioanalyzer system and the obtained RNA profiles and RIN were compared. Figure A shows representative RNA profiles of *D. melanogaster (C), A. mellifera (E), C. floridanus (F), Z. nevadensis (G), H. zea (H),* and *M. persicae (L)*. The effect of degraded insect RNA on the outcome of quantitative RT-PCR experiments was evaluated. Figure B shows degradation of total RNA samples from *D. melanogaster* at room temperature for the indicated time. Even though the RIN, developed for eukaryotic total RNA, may not be practical for all insect species, the electropherograms provide suitable information to judge RNA integrity across insect species.

Application note: 5991-7903EN

Analysis of Total RNA

Comparison of the RIN and RQN



Instrument: **Fragment Analyzer and Bioanalyzer systems**

Kit: **RNA (15 nt) kit, HS RNA (15 nt) kit (Fragment Analyzer system)**
RNA 6000 Nano kit, RNA 6000 Pico kit (Bioanalyzer system)

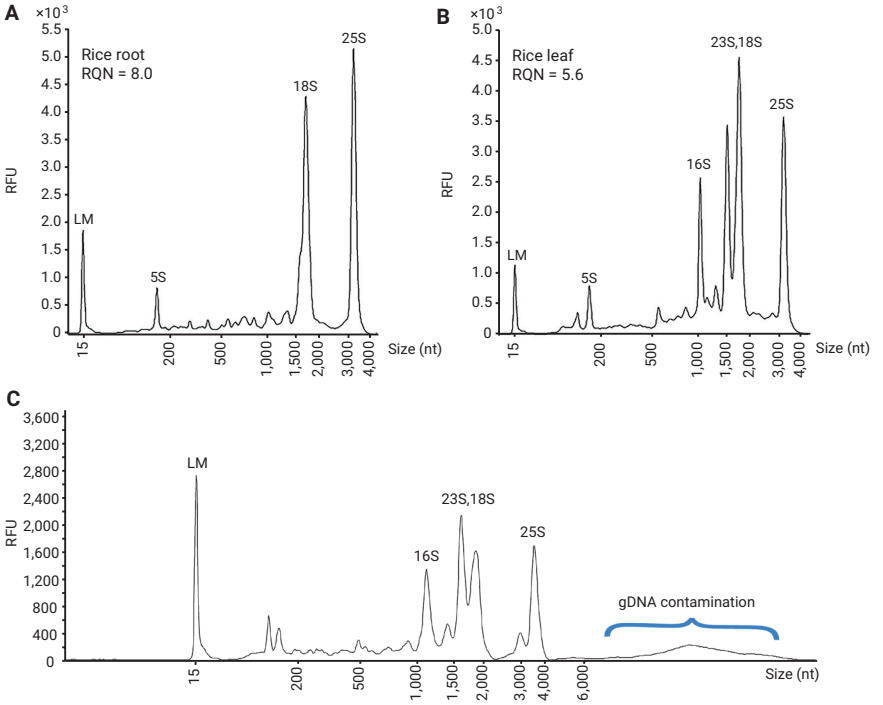
Software assay: **Eukaryote Total RNA Nano assay, Eukaryote Total RNA Pico assay (Bioanalyzer)**

Abstract: The Bioanalyzer instrument is well established for providing a reliable, automated RNA integrity number (RIN). The RIN provides an objective assessment of RNA integrity. The Fragment Analyzer offers a user-independent quality metric, the RNA quality number (RQN), for easy evaluation of total RNA quality. Both the RIN and RQN consider the entire electropherogram, with scoring from 1 to 10, where 10 indicates the highest possible RNA quality and 1 completely degraded RNA. Eukaryotic samples with a varying degree of RNA integrity, from completely intact, to mildly and strongly degraded, were compared on the Bioanalyzer and Fragment Analyzer instruments. Both the standard sensitivity (A) and High Sensitivity RNA (B) kits on both instruments provided comparable RIN and RQN scores throughout the degradation series. This is demonstrated with the slope and R^2 value close to 1. This technical overview also shows a strong correlation between the RIN and RQN for prokaryotic *E. coli* RNA samples.

Technical overview: 5994-1860EN

Analysis of Total RNA

Assessing quality of plant RNA



Instrument: **Fragment Analyzer systems**

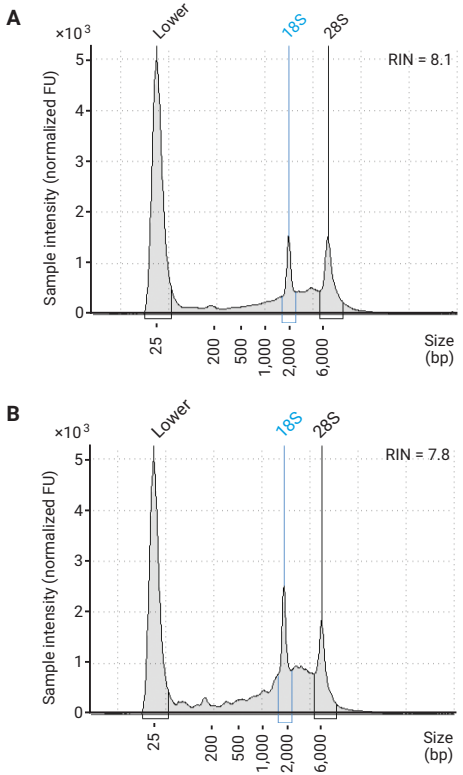
Kit: **HS RNA kit**

Abstract: Plant tissues have three types of ribosomal RNAs (rRNA): chloroplast, cytosolic, and mitochondrial. The 5200 Fragment Analyzer system delivers ample resolution to separate all four chloroplastic rRNA peaks: 16S, 23S, 18S, and 25S. The ProSize data analysis software has a dedicated plant mode for evaluating complex plant RNA samples. For example, the electropherograms pictured highlight the differences between rice root (A) and leaf RNA (B). Leaf samples have an additional chloroplast rRNA not present in root RNA. High-quality RNA, free of genomic DNA (gDNA), is critical to the success of many downstream techniques, including RT-PCR, microarray analysis, and NGS. The electropherogram displayed in the ProSize data analysis software helps determine if any gDNA contamination is present in the RNA sample. Figure C shows an example of corn leaf RNA with gDNA contamination.

Application note: 5994-0518EN

Analysis of Total RNA

RNA sequencing: QC of starting material



Instrument: **TapeStation systems**

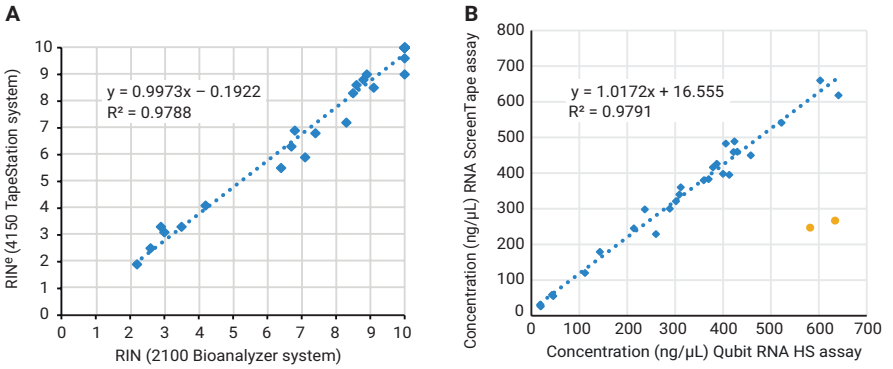
Assay: **RNA ScreenTape assay**

Abstract: RNA is sensitive to degradation due to the ubiquitous presence of RNase and its more fragile single-stranded structure. Therefore, monitoring the integrity of starting material is indispensable, and processing a reference sample as positive control throughout the library preparation and sequencing is highly advisable. With the RNA ScreenTape assay, the RNA integrity number equivalent (RIN^e) delivers an objective assessment of the integrity of RNA starting material. RIN^e is a proven equivalent to the widely accepted quality metric RIN. The fragmentation conditions of RNA-seq protocols used by the sequencing core facility are optimized for high-quality RNA; more precisely, a RIN^e of 8.0 or higher is recommended for successful library preparation. The figure shows two samples close to this threshold, one passing (A) and one failing (B) the quality requirement. The use of degraded RNA can result in low yield, over-representation of 3' ends of the RNA molecules, or failure of the protocol.

Application note: 5994-0327EN

Analysis of Total RNA

Integrity analysis and quantification of total RNA samples with the 4150 TapeStation system



Instrument: **TapeStation and Bioanalyzer systems**

Kit: **RNA ScreenTape assay (TapeStation), RNA 6000 Nano kit (Bioanalyzer)**

Software assay: **Eukaryote Total RNA Nano assay (Bioanalyzer)**

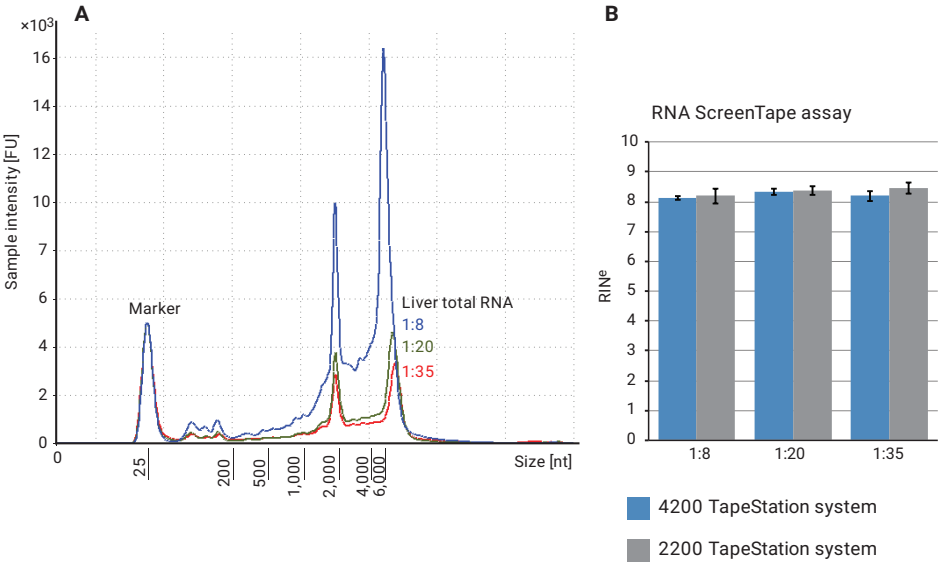
Abstract: RNA samples are often subject to degradation by RNases, chemical, or other environmental impacts. Therefore, RNA integrity analysis is a crucial step before any downstream application like NGS. The RIN^e metric from the TapeStation software is equivalent to the RIN metric provided by the RNA assays of the Bioanalyzer system. The comparative analysis of 30 RNA samples extracted from mouse cancer tissue verified that RIN and RIN^e highly correlate, with a slope of 0.997 and a goodness-of-fit (R^2) of 97.9% (A).

The same samples were quantified fluorometrically and results were compared with the RNA ScreenTape assay. Both assays resulted in a similar total RNA concentration of samples, with a slope of 1.017 and a R^2 of 97.9%, showing high correlation (B). Two degraded RNA samples were treated as outliers. Overall, the RNA ScreenTape assay provides a very useful tool in determining integrity and quantity of RNA starting material for sequencing in a single analysis step.

Application note: 5994-0946EN

Analysis of Total RNA

Equivalence of the 4200 TapeStation and 2200 TapeStation system RNA integrity number



Instrument: **TapeStation systems**

Assay: **RNA ScreenTape assay, High Sensitivity RNA ScreenTape assay**

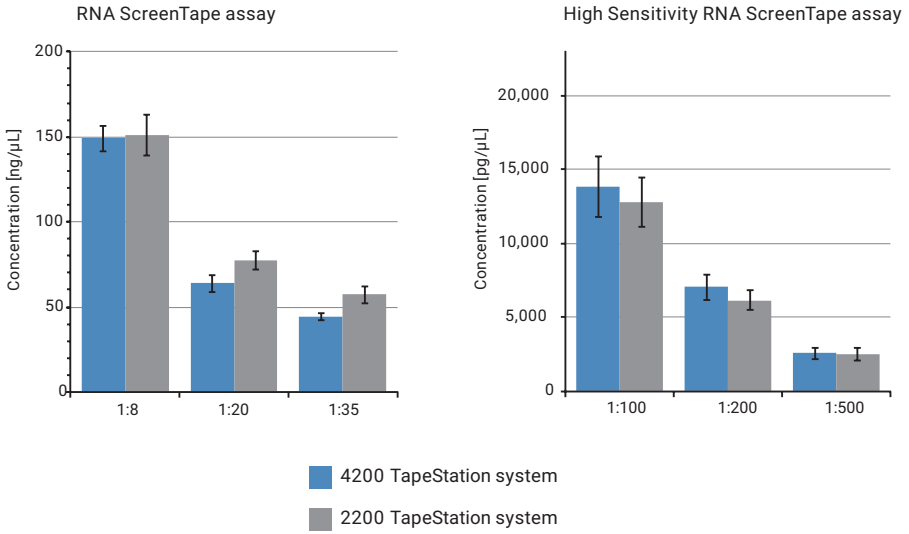
Abstract: As RNA is highly sensitive to degradation, quality control is essential, and usually includes RNA integrity analysis and quantification. The above electropherogram overlay represents different dilutions of liver total RNA analyzed on the 4200 TapeStation system with the RNA ScreenTape assay (A).

The RNA integrity number equivalent (RIN^e), indicating RNA integrity, is automatically determined by the TapeStation software. The RIN^e is calculated on a scale from 1 to 10. A high RIN^e indicates highly intact total RNA, whereas a low RIN^e corresponds to a strongly degraded sample. The bar chart (B) illustrates that RNA integrity analysis is highly reproducible and independent of the analyzed concentration for both TapeStation systems. In comparison to the 2200 TapeStation system, the 4200 TapeStation system further reduces manual operating time, enabling the analysis of 96 samples without physical intervention. This is a primary requirement for high-throughput NGS labs.

Application note: 5991-6892EN

Analysis of Total RNA

Equivalence of the 4200 TapeStation and 2200 TapeStation system quantification



Instrument: **TapeStation systems**

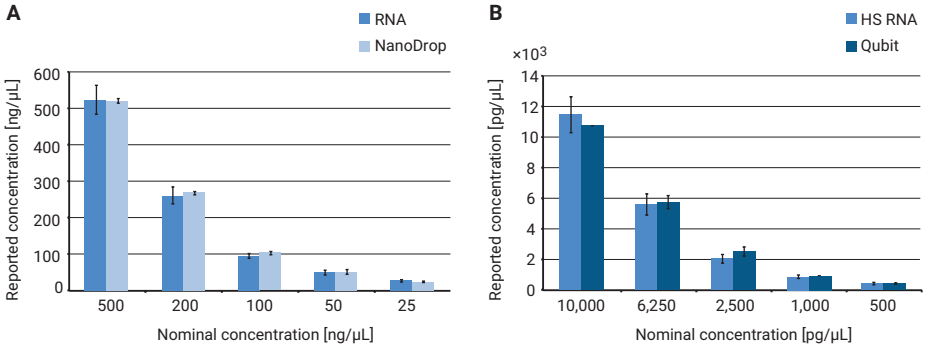
Assay: **RNA ScreenTape assay, High Sensitivity RNA ScreenTape assay**

Abstract: Various concentrations of liver total RNA were quantified with the RNA ScreenTape assay and the High Sensitivity RNA ScreenTape assay using the 4200 TapeStation system and the 2200 TapeStation system (n=15). The data demonstrates that the total RNA quantification with both TapeStation systems is comparable for the two assays. The total RNA quantification precision with the 4200 TapeStation system was below 15% coefficient of variation (CV) for the RNA and High Sensitivity RNA ScreenTape assays.

Application note: 5991-6892EN

Analysis of Total RNA

RNA quantification with the TapeStation system in comparison to spectrophotometric and fluorimetric measurements



Instrument: **TapeStation systems**

Assay: **RNA ScreenTape assay, High Sensitivity RNA ScreenTape assay**

Abstract: To determine the quantification accuracy and reproducibility of the RNA ScreenTape assay, rat kidney total RNA samples were prepared at five different nominal concentrations ranging from 25 to 500 ng/µL. This set of samples was analyzed with the RNA ScreenTape assay on the 2200 TapeStation system and the NanoDrop 2000 system by four different analysts (A). Both quantification methods, the RNA ScreenTape assay and the NanoDrop 2000 system, yielded comparable results for the tested concentrations.

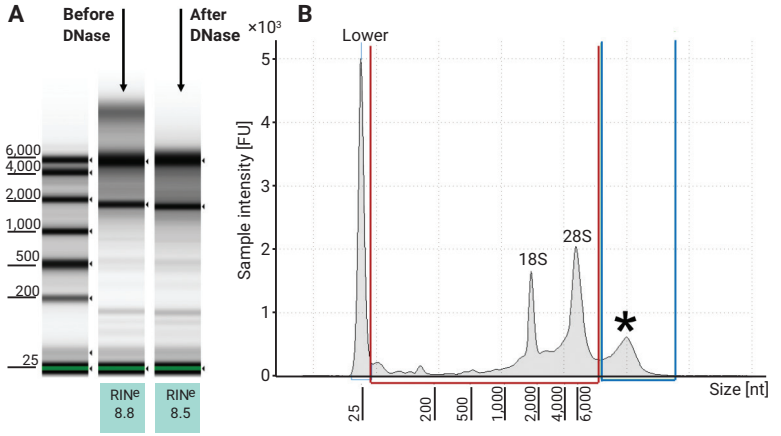
Similarly, the quantification accuracy and reproducibility of the High Sensitivity RNA ScreenTape assay were tested. Rat kidney total RNA samples were prepared at five different nominal concentrations ranging from 500 to 10,000 pg/µL. The samples were analyzed with the High Sensitivity RNA ScreenTape assay on the 2200 TapeStation system and the Qubit dsDNA HS assay (B). Both quantification methods, the High Sensitivity RNA ScreenTape assay and the Qubit 2.0 Fluorometer, yielded comparable results for the tested samples.

The data confirms that quantification results for both RNA ScreenTape assays are in good agreement to data generated by spectrophotometric and fluorometric methods.

Technical overview: 5991-3426EN

Analysis of Total RNA

RNA purity – Detection of genomic DNA contamination



NanoDrop		2200 TapeStation system				
Total conc. (ng/µL)	RNA purity (%)	Total conc. (ng/µL)	RNA region conc. (ng/µL)	gDNA region conc. (ng/µL)	RNA purity (%)	Purity accuracy (%)
290	69.0	276	175	98	63.4	91.9
275	72.7	321	221	95	68.8	94.7
250	80.0	246	177	64	72.0	89.9
225	88.9	237	198	37	83.5	94.0
210	95.2	194	175	16	90.2	94.7

Instrument: **TapeStation systems**

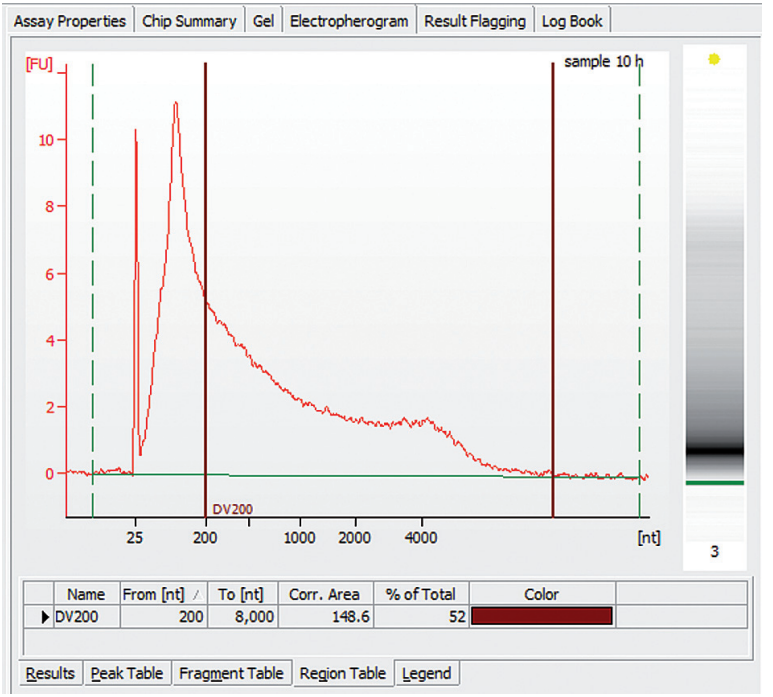
Assay: **RNA ScreenTape assay**

Abstract: During RNA purification procedures, residual genomic DNA may be present. This can lead to inaccurate RNA quantification or cause issues with downstream applications. The identification of genomic DNA contamination is therefore useful in deciding whether further cleanup of the extracted RNA is required. In contrast to some capillary based systems, the TapeStation system can resolve intact genomic DNA contaminants from the large ribosomal RNA. The figure shows the analysis of rat kidney total RNA spiked with mouse genomic DNA. The genomic DNA contamination results in an additional peak running above the 28S rRNA peak (*). Sample analysis after DNase treatment reveals that the corresponding band in the gel image (A) and peak in the electropherogram (B) disappears.

Technical overview: 5991-3426EN

Analysis of FFPE RNA

Quality control with DV_{200} evaluation of FFPE RNA samples



Instrument: **2100 Bioanalyzer system**

Kit: **RNA 6000 Nano kit, RNA 6000 Pico kit**

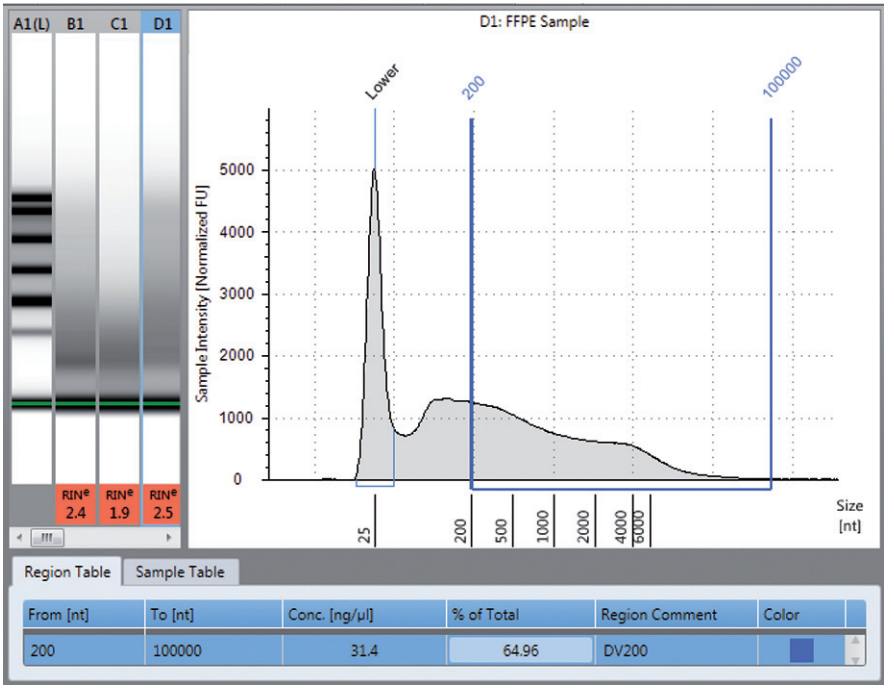
Software assay: **DV200 RNA Nano assay, DV200 RNA Pico assay**

Abstract: With the DV_{200} metric, degraded RNA samples extracted from FFPE tissue can be classified according to their size distribution. This technical overview describes the simplified evaluation of the DV_{200} using the DV200 RNA Nano and DV200 RNA Pico assays with the Bioanalyzer system. The assays can be used for re-analysis of existing data files as well as for new data acquisitions. The DV200 RNA assays automatically define a region from 200 to 8,000 nucleotides, as shown in the figure. A corresponding DV_{200} is provided in the Region Table in the column % of Total. The DV_{200} results can be saved, exported, and displayed in reports. Samples with RIN <4 are flagged in different colors for easy assessment of the DV_{200} range.

Technical overview: 5991-8287EN

Analysis of FFPE RNA

DV_{200} evaluation with RNA ScreenTape assays



Instrument: **TapeStation systems**

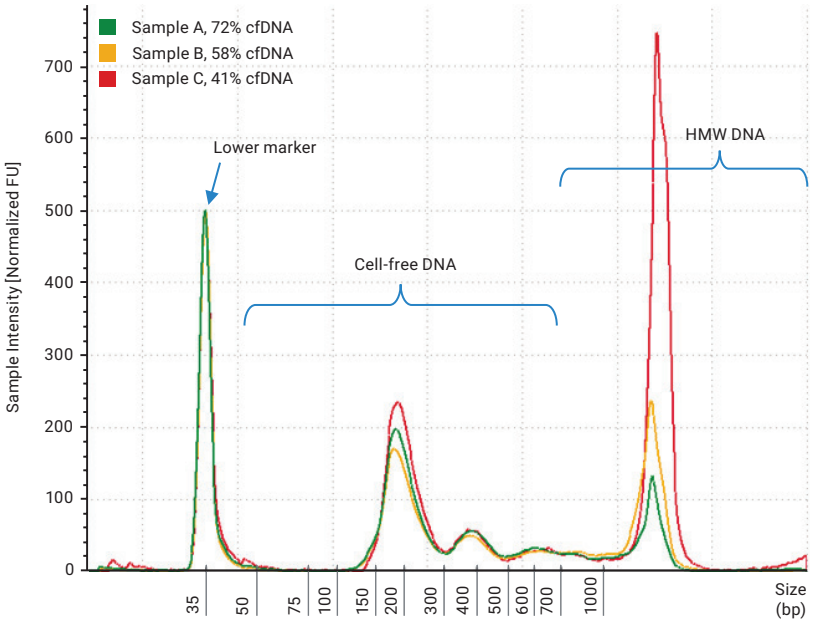
Assay: **RNA ScreenTape assay, High Sensitivity RNA ScreenTape assay**

Abstract: The DV_{200} quality metric represents the percentage of RNA fragments above 200 nucleotides and shows a high correlation to the precapture library yield of RNA samples originating from formalin-fixed paraffin-embedded (FFPE) tissue. The RNA and High Sensitivity RNA ScreenTape assays enable fast and easy analysis of FFPE RNA samples, with the TapeStation software displaying DV_{200} results after region setup as percentage of total. DV_{200} region setup can be automated for repeated FFPE RNA sample analysis and all region data can be exported and reported. Both the RNA ScreenTape assay and High Sensitivity RNA ScreenTape assay yielded highly comparable results.

Application note: 5991-8355EN

Analysis of Cell-free DNA

%cfDNA quality metric for the TapeStation systems



Instrument: **TapeStation systems**

Assay: **Cell-free DNA ScreenTape assay**

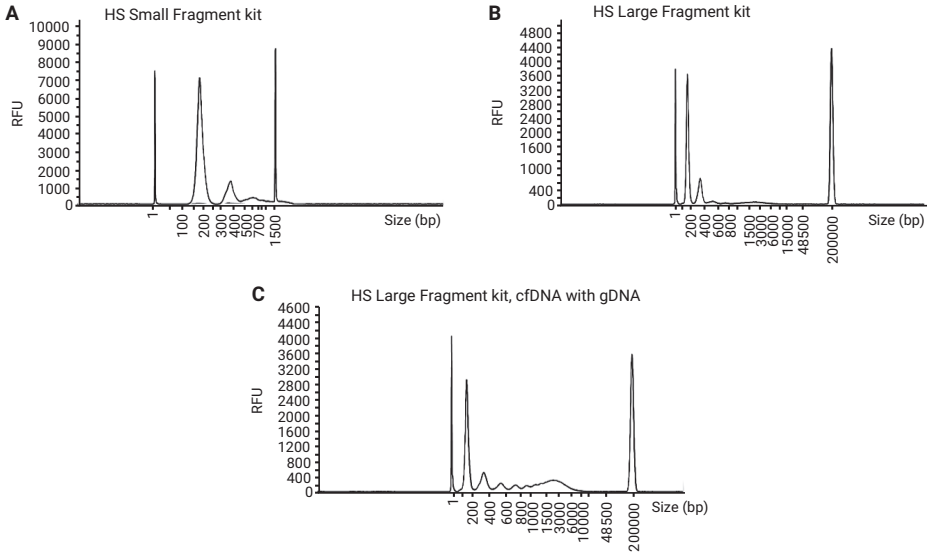
Abstract: Cell-free DNA samples separated by automated electrophoresis with the Cell-free DNA ScreenTape assay display a prominent peak at approximately 170 bp, which represents the mononucleosome DNA fragments. The mononucleosome peak is followed by nucleosome multimers containing less abundant DNA fragments. Some samples may also display HMW DNA above 700 bp.

During traditional NGS workflows, the presence of HMW DNA can negatively affect library yield and sequencing quality. The Cell-free DNA ScreenTape assay features a new quality metric, %cfDNA, reflecting the percentage of cfDNA subcomponents that are present in the preset region between 50 and 700 bp, in relation to the total sample DNA. The %cfDNA metric allows the user to evaluate sample quality and identify whether a sample contains sufficient cfDNA for downstream processes. The figure displays the electropherogram profiles of three samples with different %cfDNA values. The samples are composed of comparable amounts of cfDNA subcomponents but different HMW DNA quantity, resulting in different quality levels.

Technical overview: 5994-1390EN

Analysis of Cell-free DNA

Quality control analysis of cfDNA



Instrument: **Fragment Analyzer systems**

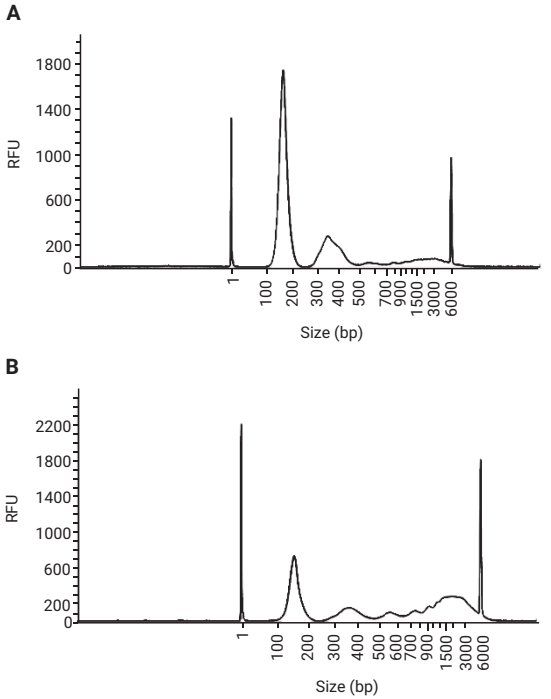
Kit: **HS Small Fragment kit, HS Large Fragment kit**

Abstract: Circulating cell-free DNA (cfDNA) is gaining prevalence as a noninvasive, alternative approach for the detection of tumor mutations in cancer management and screening tests for fetal abnormalities from the mother's blood. cfDNA is known to circulate in healthy and pathological conditions and is present in plasma, serum, cerebral spinal fluid, and saliva. A typical cfDNA electropherogram displays one, two, or three nucleosomal fragments. In this example, the HS Small Fragment kit distinctively separates three cfDNA peaks from healthy human serum at 166 bp, 353 bp, and 569 bp (A). These peak sizes correspond to a nucleosome-guided fragmentation pattern of apoptotic cfDNA, often referred to as mononucleosome, dinucleosome, and trinucleosome cfDNA. The same sample was also analyzed with the HS Large Fragment kit and three peaks were separated at 157 bp, 347 bp, and 528 bp (B). The HS Large Fragment kit also enables reliable quantification and sizing for cfDNA samples containing fragmented genomic DNA (C).

Application note: 5994-0510EN

Analysis of Cell-free DNA

Separation of cfDNA with the HS NGS Fragment kit



Instrument: **Fragment Analyzer systems**

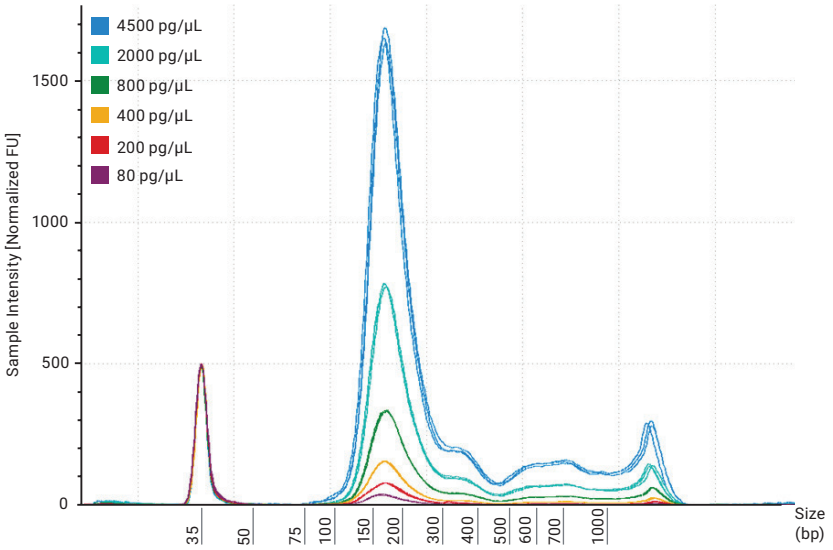
Kit: **HS NGS Fragment kit (1-6000 bp)**

Abstract: Quality analysis of extracted circulating cell-free DNA (cfDNA) plays an important role in determining sizing and purity, providing necessary knowledge for sensitive downstream applications such as NGS. cfDNA was extracted from healthy human serum using the Quick-cfDNA serum and plasma kit from Zymo. Typical cfDNA separation profiles display two or three distinct peaks (A). Extraction of fragmented genomic DNA (gDNA) with cfDNA can occur. The results showed that the HS NGS Fragment kit (1-6000 bp) was able to effectively separate the three nucleosome cfDNA peaks from gDNA (B). Some extraction kits have the option to use carrier RNA during cfDNA extraction. Carrier RNA was shown to comigrate with the dinucleosome peak when analyzed using the HS NGS Fragment kit, inflating the concentration of the second peak. Taking into consideration the cfDNA extraction methods when analyzing cfDNA QC is recommended.

Application note: 5994-0522EN

Analysis of Cell-free DNA

Characterization of cell-free DNA



Instrument: **TapeStation systems**

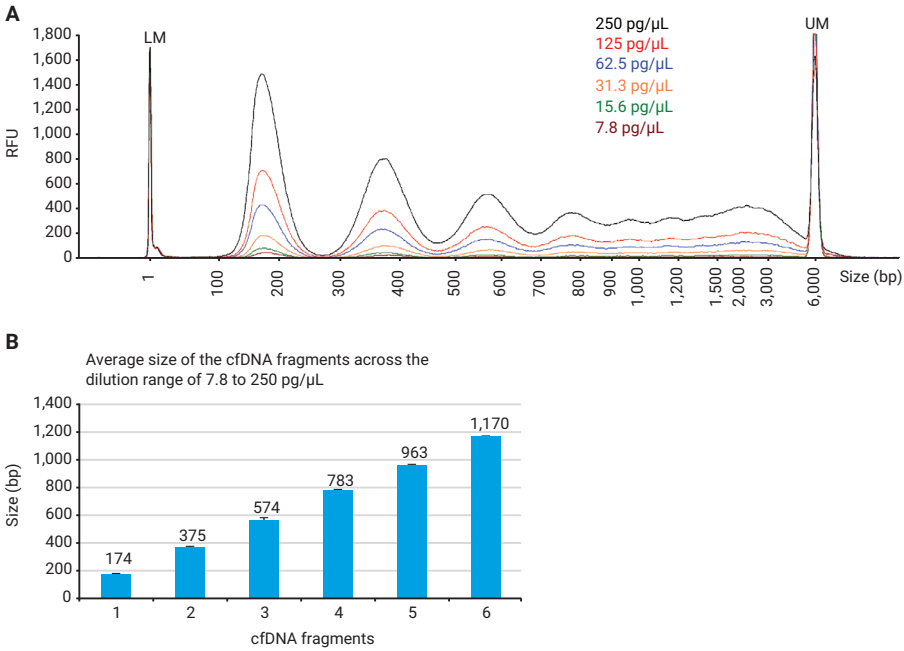
Assay: **Cell-free DNA ScreenTape assay**

Abstract: To demonstrate the concentration independence of the %cfDNA metric, a dilution series of a reference cfDNA sample (n=24), covering the entire concentration range of the assay, was analyzed. The average %cfDNA value was 85.0% ± 1.0 with a minimum value of 83.7% and maximum value of 86.6%. The %cfDNA results and the electropherogram profiles were consistent over the entire concentration range of the assay. In the figure above, each concentration is overlaid with three replicates to demonstrate the consistency and precision of the Cell-free DNA ScreenTape assay. These results show that the %cfDNA quality metric provided by the Cell-free DNA ScreenTape assay is highly accurate and precise, and that the percentage is independent of the sample concentration.

Technical overview: 5994-1390EN

Analysis of Cell-free DNA

Separation of low concentrated cfDNA



Instrument: **Femto Pulse system**

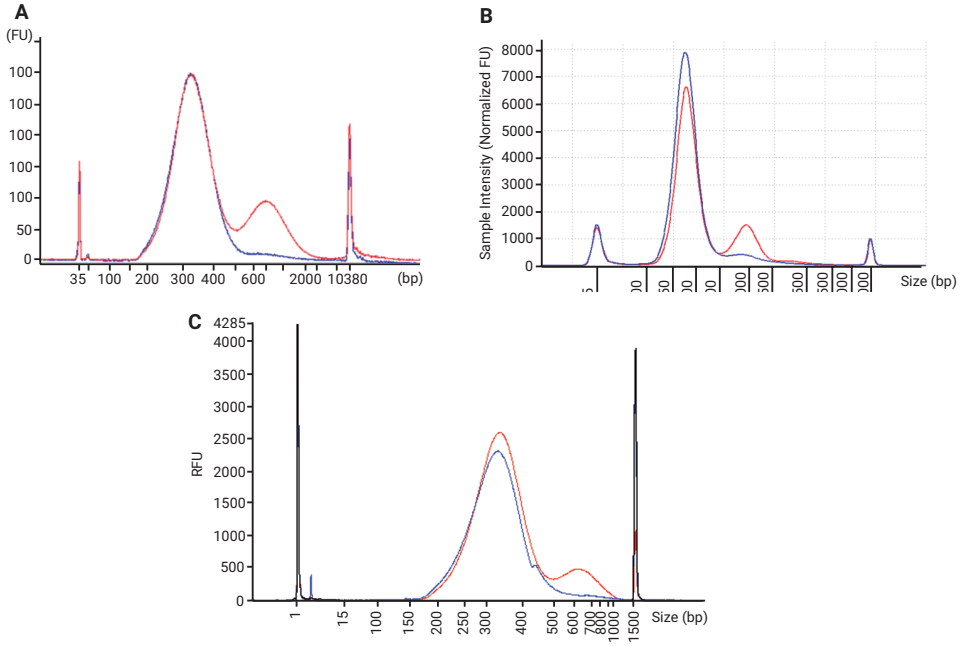
Kit: **Ultra Sensitivity NGS kit**

Abstract: The Femto Pulse system offers unparalleled sensitivity for the analysis of low concentrated cfDNA samples. In addition, the system's high resolution provided allows samples with multiple fragments to have complete separation between peaks. cfDNA samples can differ in the number of fragments present, but the mono- and dinucleosome fragments are usually both present. cfDNA analyzed on the Femto Pulse with the Ultra Sensitivity NGS kit displayed six fragments (A). In the dilution series (250 to 7.8 pg/µL), the first three cfDNA fragments were completely separated at all concentrations, with the fourth fragment easily distinguishable down to 15.6 pg/µL. The fifth and sixth fragment peaks were less apparent below 31.3 pg/µL. A seventh fragment peak was observed only in the highly concentrated samples, 62.5 pg/µL and higher. Sizing remained consistent for all six peaks throughout the concentration range they were visible for (B).

Application note: 5994-0514EN

Next-Generation Sequencing

Quality control of NGS libraries with daisy chains



Instrument: **Bioanalyzer, TapeStation, and Fragment Analyzer systems**

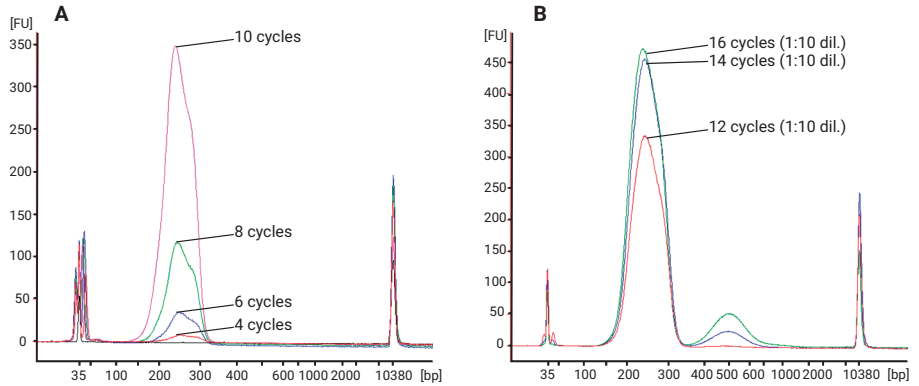
Kit: **High Sensitivity DNA kit (Bioanalyzer), High Sensitivity D5000 ScreenTape assay (TapeStation), HS Small Fragment kit (Fragment Analyzer)**

Abstract: The Agilent automated electrophoresis solutions offer fast and reliable quality control of NGS libraries. The Bioanalyzer, TapeStation, and Fragment Analyzer systems with dedicated high sensitivity assays allow unambiguous detection of daisy chains and accurate sizing of the target library peak. Two KAPA HyperPlus libraries with different levels of amplification were analyzed on the Bioanalyzer (A), TapeStation (B), and Fragment Analyzer (C) systems using the High Sensitivity DNA kit, High Sensitivity D5000 ScreenTape assay, and HS Small Fragment kit respectively. As shown in the overlays, both libraries (blue – library 1, red – library 2) contained the desired library peak with a pronounced secondary peak in library 2. The excessive amplification of library 2 resulted in the formation of daisy chains, which were observed as an additional higher molecular weight peak in all electropherograms. The daisy chains migrated slower through the gel matrix and were easily detected by all Agilent automated electrophoresis instruments using the respective assays. In this application note, we provide a recommendation for which assay to employ to reliably visualize daisy chain products in next-generation sequencing libraries. Furthermore, we emphasize the consistency between the instruments and reproducibility of analysis confirmed by results on a series of double dilutions.

Application note: 5994-2233EN

Next-Generation Sequencing

DNA library QC in target enrichment and NGS workflows



From [bp]	To [bp]	Corrected Area	% of Total	Average Size [bp]	Size Distribution in CV [%]	Concentration [pg/µL]	Molarity [pmol/L]
100	2,000	395.8	51	254	12.4	283.33	1,713.8

Quantitation after four PCR cycles.

Instrument: **2100 Bioanalyzer system**

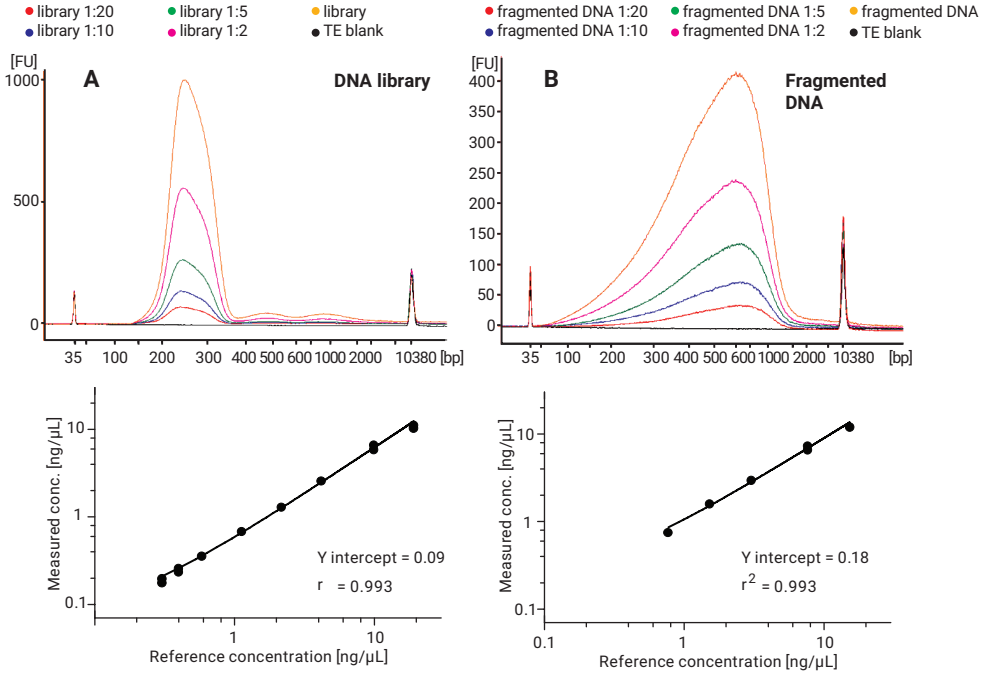
Kit: **High Sensitivity DNA kit**

Abstract: The High Sensitivity DNA kit was used for quality control of amplified and purified DNA samples from the post-hybridization PCR amplification step before sequencing, during the SureSelect Target Enrichment workflow. The electropherograms of typical PCR-amplified DNA libraries show a smear from 150 to 350 nucleotides (A). Figure B shows that we can see that the quality of the PCR product depends on the number of PCR cycles performed. After 14 PCR cycles, an additional DNA smear at approximately 500 bp was detected in the electropherogram. The highly sensitive nature of the High Sensitivity DNA kit allowed the amplified DNA to be detected and reliably quantified, even after only four PCR cycles. The number of library PCR cycles can therefore be reduced, removing amplification bias and significantly improving the data quality with increased accuracy.

Application note: 5990-5008EN

Next-Generation Sequencing

Sizing and quantification of DNA libraries and fragmented DNA



Instrument: **2100 Bioanalyzer system**

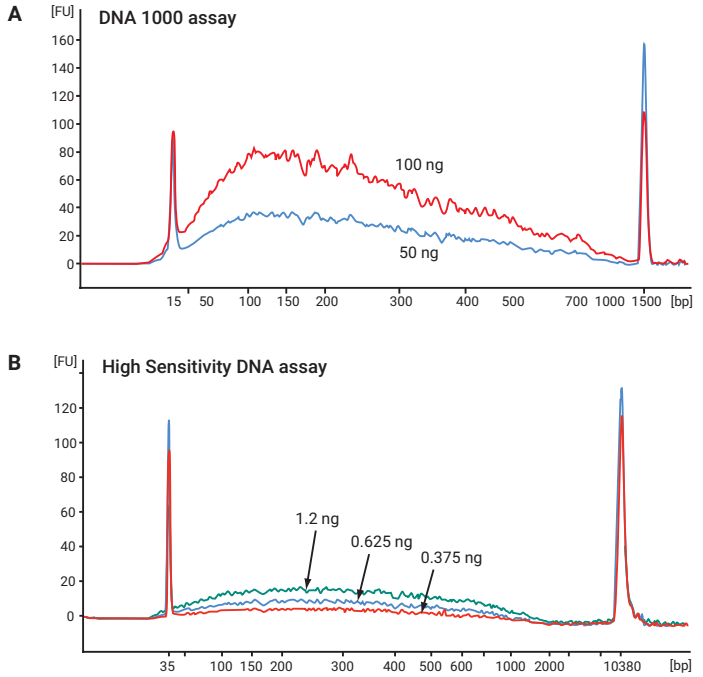
Kit: **High Sensitivity DNA kit**

Abstract: The High Sensitivity DNA kit provides sizing and quantification of DNA fragments and DNA smears in the 50 to 7,000 bp size range, down to pg/µL sensitivity. This is especially useful for sample quality control and monitoring critical steps in NGS workflows, including DNA fragmentation, target enrichment, and DNA library amplification. The analysis of a dilution series from two typical NGS samples, Illumina DNA library (A) and fragmented DNA (B) was performed. For both DNA sample types, the double logarithmic plot demonstrates an excellent linearity with $r^2 = 0.993$. The linear dynamic range for smear samples of the High Sensitivity DNA kit was found to be between 50 to 100 pg/µL and 5,000 to 10,000 pg/µL. This linear dynamic range depends on the library type and fragment distribution. The broad linear dynamic range of the High Sensitivity DNA kit enables the detection of less abundant products, such as PCR artifacts and impurities.

Technical overview: 5990-4417EN

Next-Generation Sequencing

Analysis of limited DNA material on the Pippin Prep system



Instrument: **2100 Bioanalyzer system**

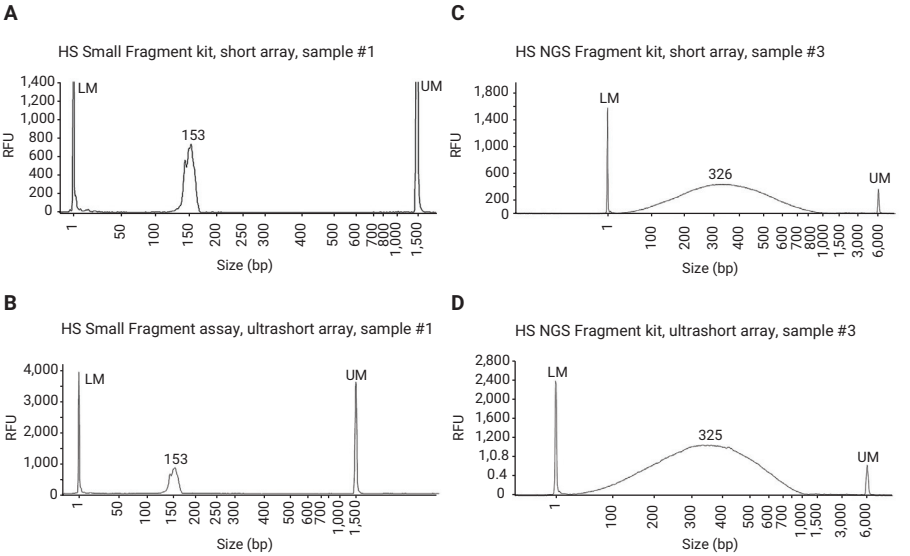
Kit: **DNA 1000 kit, High Sensitivity DNA kit**

Abstract: The Agilent 2100 Bioanalyzer system with the High Sensitivity DNA kit complement the Pippin Prep automated size selection workflow (Sage Science, Inc.). Figure A demonstrates the DNA analysis of a restriction digest of *E. coli* genomic DNA to simulate a sheared Pippin Prep input sample. Figure B shows that the High Sensitivity DNA kit requires only a small amount of DNA (1.2 ng) to achieve a roughly equivalent signal to 50 ng on the DNA 1000 kit. Even smaller DNA amounts (0.375 ng) yield reasonable electropherograms showing input size distribution. This allows tailoring fractionation settings for the Pippin Prep system, maximizing chances for successful library construction. Afterwards, the 2100 Bioanalyzer system can be used to confirm size ranges, quality, and purity of the Pippin Prep processed samples. The 2100 Bioanalyzer and Pippin Prep systems work well in combination, increasing the efficiency of the NGS library preparation process.

Application note: 5990-8382EN

Next-Generation Sequencing

Library size and quantification comparison with two kits on the Fragment Analyzer systems



Instrument: **Fragment Analyzer systems**

Kit: **HS Small Fragment kit, HS NGS Fragment kit (1-6000 bp)**

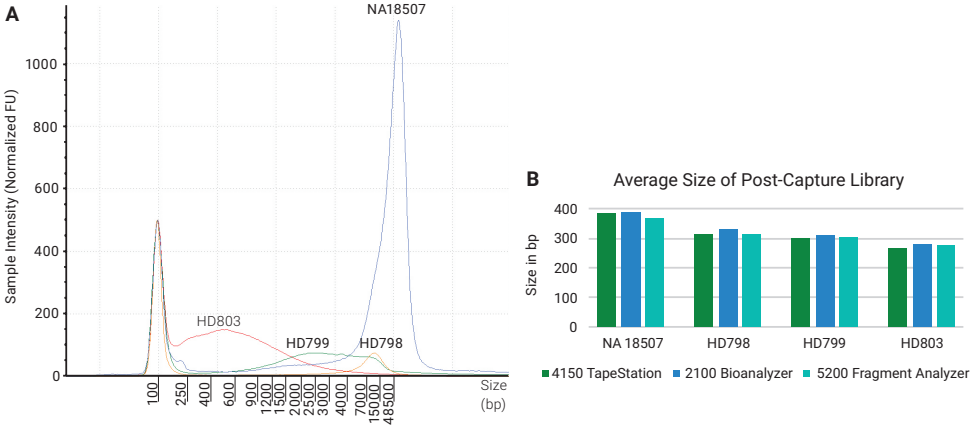
Software assay: **DNF-477-22-HS Small Fragment assay, DNF-477-33-HS Small Fragment assay, DNF-474-22-HS NGS Fragment assay, DNF-474-33-HS NGS Fragment assay**

Abstract: The quality of NGS libraries is crucial to successful sequencing results. The Fragment Analyzer systems offers easy analysis of sheared genomic DNA (gDNA) and libraries with the HS NGS Fragment kit (1-6000 bp) and the HS Small Fragment kit. The HS NGS Fragment kit (C and D) analyzes larger smears and fragments up to 6,000 bp, while the HS Small Fragment kit (A and B) focuses on smaller sizes up to 1,500 bp. The FA 12-Capillary Array Ultrashort (22 cm) decreases run time by 10 to 20 minutes compared to the standard FA 12-Capillary Array Short, 33 cm. The size and concentration of several DNA smears were compared between both kits and the short and ultrashort arrays. Library sizing and quantification remained consistent between the short (A and C) and ultrashort (B and D) arrays and the two kits. The HS Small Fragment kit and the HS NGS Fragment kit can be used interchangeably for sizing and quantification of NGS libraries, as long as the sample fits within the sizing range of the kit.

Application note: 5994-0515EN

Next-Generation Sequencing

Quality control in the Magnis SureSelect XT HS workflow



Instrument: **Magnis NGS Prep, TapeStation, Bioanalyzer and Fragment Analyzer systems**

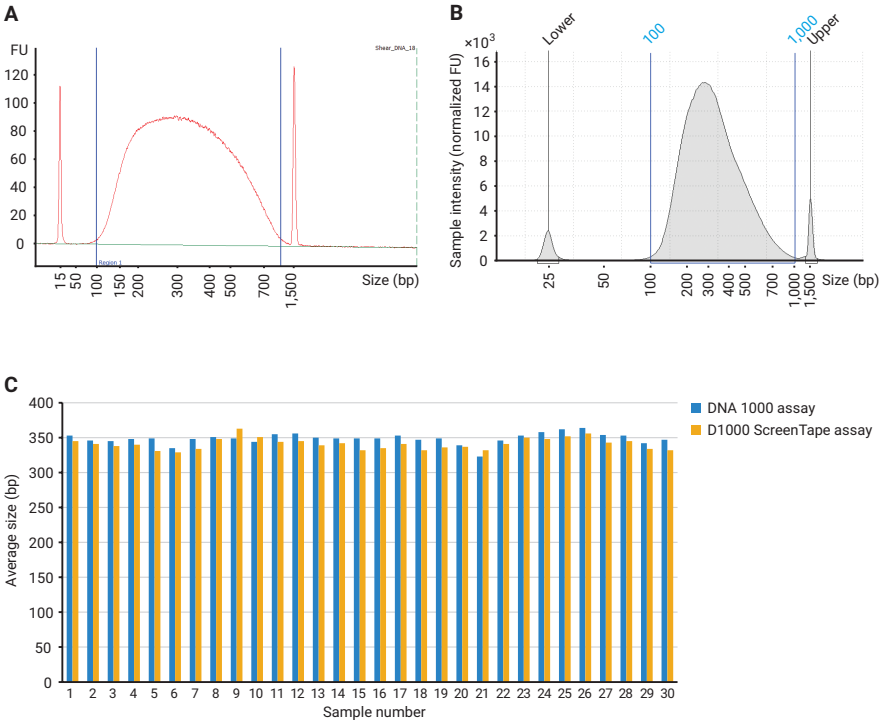
Kit: **Genomic DNA assay, HS D1000 assays (TapeStation),**
High Sensitivity DNA kit (Bioanalyzer),
HS NGS Fragment kit (1-6000 bp) (Fragment Analyzer)

Abstract: The Magnis NGS Prep system is an automated NGS library preparation solution for the SureSelect XT HS system. It addresses challenges of manual library preparation, such as hands-on time, expertise, optimization, and validation for diverse applications. Performing QC steps and quantification on the starting material, the materials derived from intermediate steps (optional), and the final library is beneficial in ensuring reliability and overall success of the sequencing data. QC steps can be performed with the automated electrophoresis portfolio of instruments, including the Bioanalyzer, TapeStation, and Fragment Analyzer systems. gDNA was assessed with the Genomic DNA ScreenTape assay on the 4150 TapeStation system for overall integrity and size in high-quality gDNA, and mildly, moderately, and highly degraded FFPE samples (A). This assay applies a quality score, the DNA integrity number (DIN), to each sample. The score is used to optimize the fragmentation step and determine the amount of input DNA to be used in library preparation. Post-capture libraries were assessed on all three platforms and displayed similar sizing (B). All automated electrophoresis instruments provide reliable QC analysis, which is critical to ensuring successful library preparation and sequencing.

Application note: 5994-1741EN

Next-Generation Sequencing

Characterization of sheared DNA



Instrument: **TapeStation and Bioanalyzer systems**

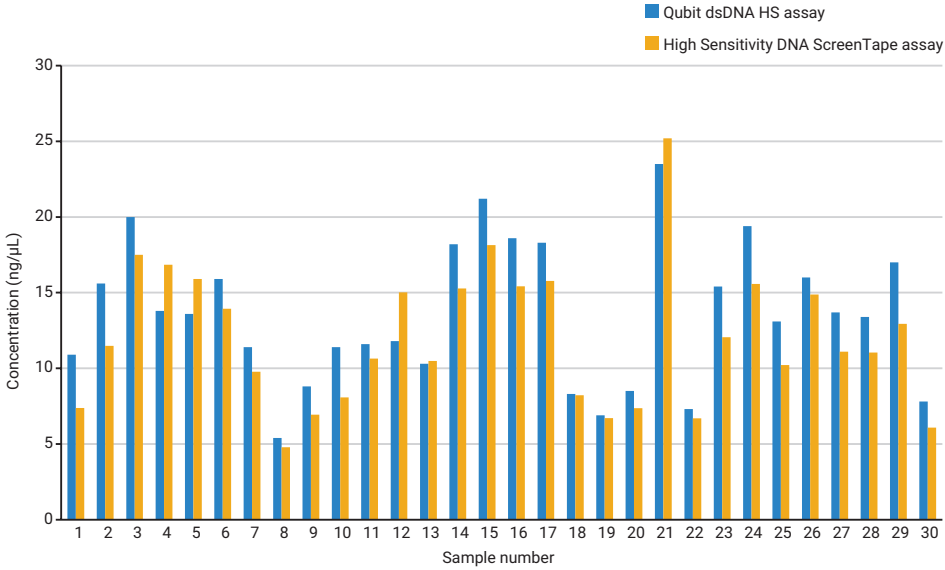
Kit: **D1000 ScreenTape assay (TapeStation), DNA 1000 kit (Bioanalyzer)**

Abstract: Frequently, the first step of a library preparation protocol is the fragmentation of gDNA by shearing with an ultrasonicator. Optimal shearing in NGS workflows can be verified with by evaluating the size distribution and electropherogram pattern of fragmented DNA samples using the 2100 Bioanalyzer (A) and the 4150 TapeStation systems (B) with the DNA 1000 kit and D1000 ScreenTape assays, respectively. Electropherograms of sheared DNA in this example display an even size distribution with no undesirable shouldering. The fragmented DNA samples show a maximum peak size between 260 and 310 bp on both systems, verifying optimal shearing (C). The size of the sample at this QC step can be compared to the size of sample after adapter ligation in the library preparation workflow, at which point a shift in size is expected. Overall sizing results of the 2100 Bioanalyzer and the 4150 TapeStation systems correlated highly with an average deviation of 2.2% for all 30 samples analyzed.

Application note: 5994-0946EN

Next-Generation Sequencing

Quantification of final NGS libraries



Instrument: **TapeStation systems**

Assay: **High Sensitivity D1000 ScreenTape assay**

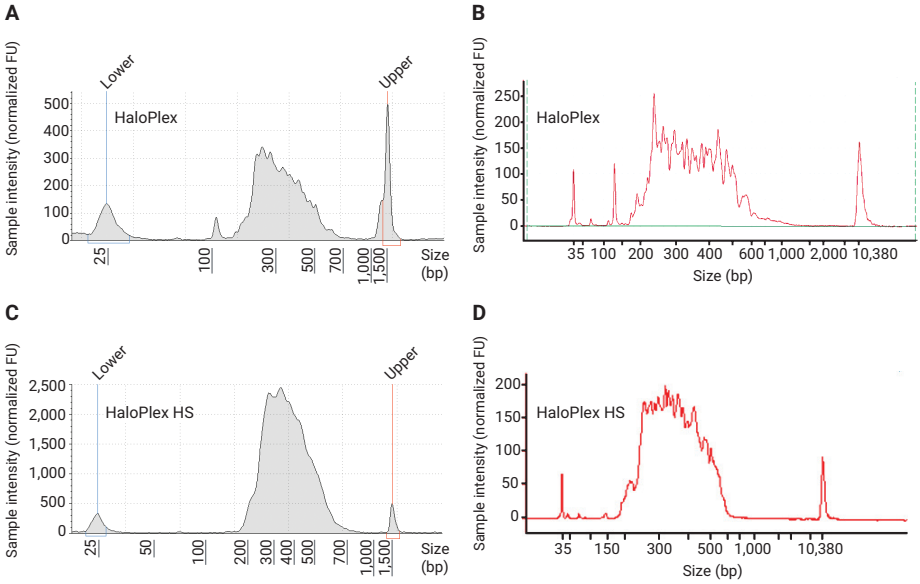
Abstract: Even read distribution during sequencing requires NGS library normalization. For this, accurate quantification as well as sizing is mandatory. The concentration data of 30 NGS libraries provided by the High Sensitivity D1000 ScreenTape assay was compared with quantification results attained from the Qubit dsDNA HS assay. The quantification using the High Sensitivity D1000 ScreenTape assay was highly comparable to the fluorometric results, with an average deviation of 11.1%.

The 4150 TapeStation system and the ScreenTape assay portfolio can therefore be used as a QC tool for quantification during library preparation.

Application note: 5994-0946EN

Next-Generation Sequencing

Quality control of HaloPlex libraries



Instrument: **TapeStation and Bioanalyzer systems**

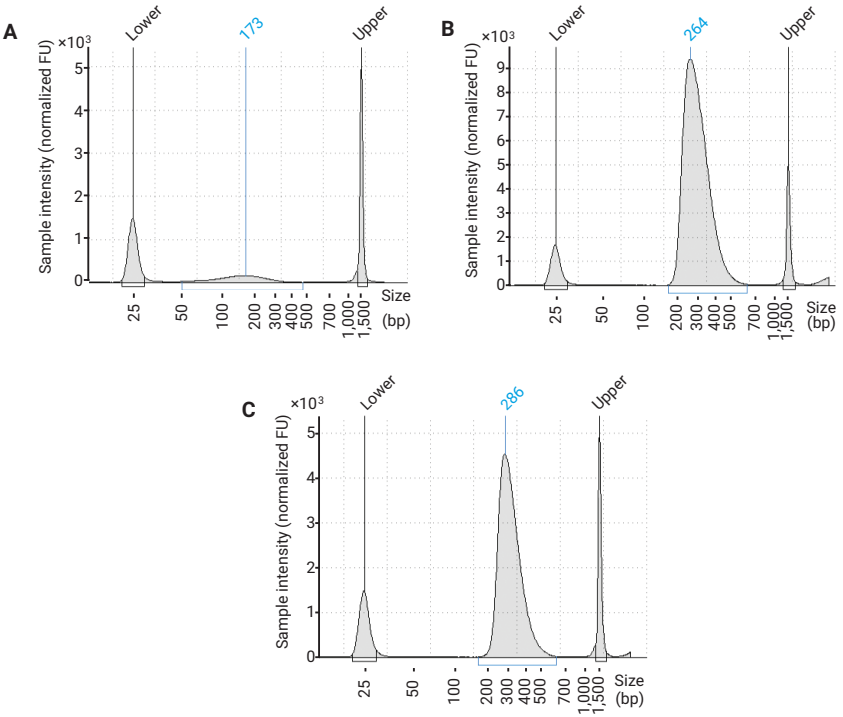
Kit: **High Sensitivity D1000 ScreenTape assay (TapeStation), High Sensitivity DNA kit (Bioanalyzer)**

Abstract: HaloPlex and HaloPlex HS target enrichment technology uses an amplicon-based approach. The final libraries of HaloPlex and HaloPlex HS workflows show a profile with a characteristic smear in the range of 175 to 625 bp, (TapeStation electropherogram (A) and Bioanalyzer electropherogram (B)), and 190 to 545 bp respectively (TapeStation electropherogram (C) and Bioanalyzer electropherogram (D)). The appearance of the profile may vary due to specific library designs and the overall quality of the input material. The electropherogram should be checked for the presence of artefactual peaks with sizes less than 150 bp, as these are related to primer dimers that can cluster and consume sequencing capacity. If the primer dimer peak is greater than 10% of the total product, an additional cleanup step with AMPure beads is recommended.

Application note: 5994-0127EN

Next-Generation Sequencing

Quality control of NGS libraries during the SureSelect XT workflow



Instrument: **TapeStation systems**

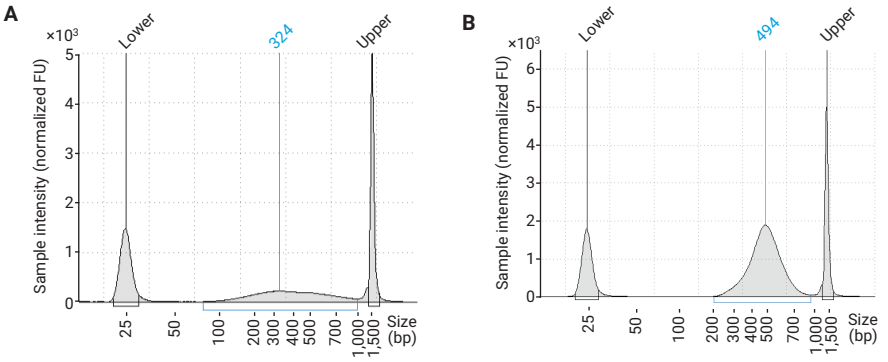
Assay: **D1000 ScreenTape assay**

Abstract: NGS target enrichment enables a detailed analysis of specific regions to identify causal genetic variants of complex conditions. The SureSelect XT protocol is designed to create libraries with enriched targeted regions of the genome for sequencing with Illumina platforms. The two intermediate QC steps include evaluation of a smear size after shearing (A) and before capturing (B). These steps can be carried out using the D1000 ScreenTape assay. The expected size range of the maximum peak of sheared DNA is 150 to 200 bp. For precapture library, a larger maximum peak size of 225 to 275 bp is expected due to adapter ligation. The last QC step qualifies the final library before pooling (C). Another size shift is expected as a result of adding index sequences. The peak maximum of the final library is expected to be between 250 and 350 bp. A minimum concentration of 2 ng/µL is expected for successfully generated final libraries.

Application note: 5994-0327EN

Next-Generation Sequencing

Chromatin immunoprecipitation (ChIP) sequencing



Instrument: **TapeStation systems**

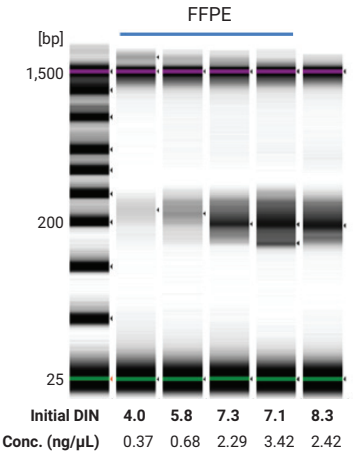
Assay: **D1000 ScreenTape assay**

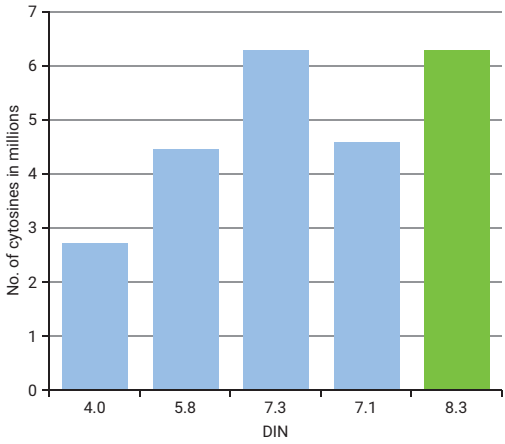
Abstract: Chromatin immunoprecipitation (ChIP) sequencing is an NGS method combining ChIP with massive parallel sequencing to reveal binding sites of DNA-associated proteins. Typically, the starting material for the ChIP-Seq workflow is chromatin-immunoprecipitated DNA, unlinked from proteins. The workflow requires two QC steps, the first being a quality assessment of the unlinked DNA, and the second the quality assessment of the final library. After initial QC of the unlinked DNA starting material (A), the samples are end-repaired, followed by dA-tailing and adaptor ligation. The adapter-ligated libraries then undergo a bead-based size selection. The size of the starting material determined in the first QC step (A) is used to collate samples to the closest available fragment length for the size selection step. End products of the ChIP workflow are analyzed with the D1000 ScreenTape assay to verify the expected total library size according to the size selection step (B). Figure B shows an example of a final library at 494 bp, generated from starting material with a size of approximately 300 bp. Larger libraries may show an increased size shift, since the bead-based size selection is not as accurate. The sizing result is used to calculate the molarity of the libraries, which are then normalized, pooled, and sequenced.

Application note: 5994-0327EN

Next-Generation Sequencing

Impact of gDNA integrity on the outcome of DNA methylation studies





Instrument: **TapeStation systems**

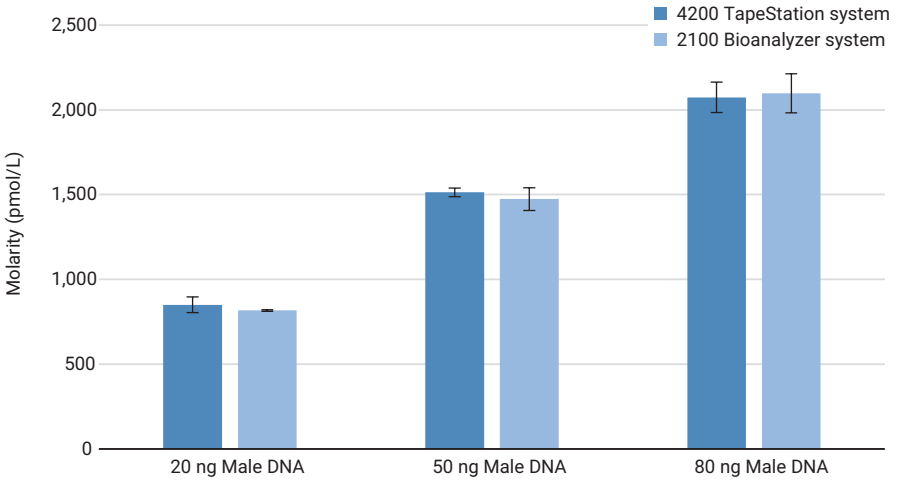
Assay: **Genomic DNA ScreenTape assay**

Abstract: Human brain tissue from a single donor was used for the genome-wide DNA methylation studies. Genomic DNA was extracted from fresh frozen tissue and used as the control. Four tissue samples were subjected to FFPE using different paraffin treatment time to generate varying levels of DNA degradation. The gDNA integrity of this starting material was determined using the Genomic DNA ScreenTape assay and the DNA integrity number (DIN). To investigate the correlation between DIN and the quality of the sequencing results, the number of covered CpG sites was determined and compared to the DIN of the initial gDNA samples. The bar chart shows that the total number of covered CpG sites varies, depending on initial DIN value of the gDNA samples. The DIN can be used as quality criterion to determine how to handle individual gDNA samples for downstream workflows, and to ensure successful DNA methylation analysis.

Application note: 5991-6427EN

Next-Generation Sequencing

Quantification of amplified SureSelect QXT gDNA libraries



Starting material		Average size (bp)		Region molarity (pmol/L)	
		4200 TapeStation system	2100 Bioanalyzer system	4200 TapeStation system	2100 Bioanalyzer system
20 ng	mean	519	533	850	817
	%CV	1.2	2.8	5.4	0.6
50 ng	mean	849	868	1513	1473
	%CV	0.6	1.4	1.7	4.5
80 ng	mean	1065	1157	2073	2097
	%CV	3.8	1.1	4.3	5.5

Instrument: **TapeStation and Bioanalyzer systems**

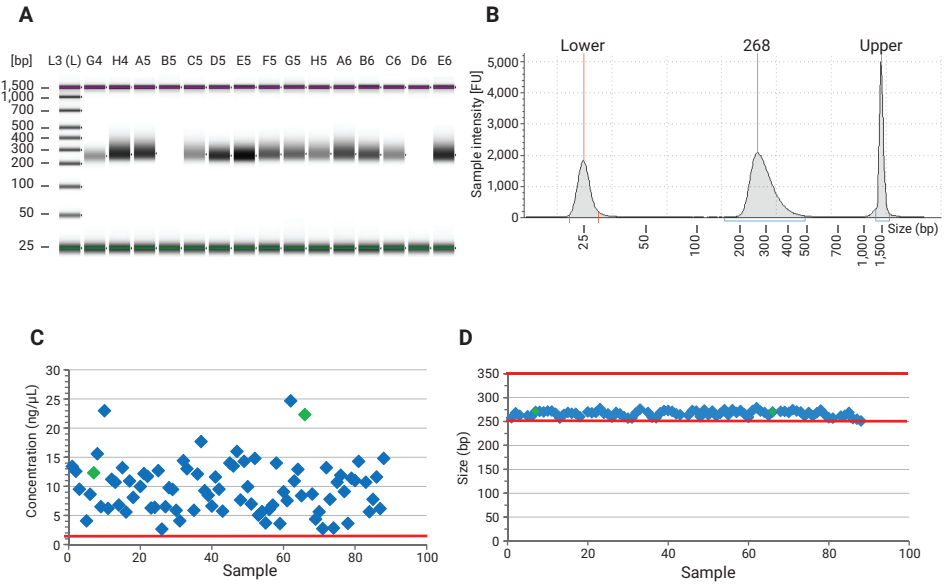
Assay: **High Sensitivity D5000 ScreenTape assay (TapeStation), DNA High Sensitivity Kit (Bioanalyzer)**

Abstract: For multiplex sequencing, SureSelect QXT whole-genome libraries are pooled so that each index-tagged sample is present in equimolar amounts in the final pool. The 4200 TapeStation and 2100 Bioanalyzer systems provide molarity and quantification data along with the sizing information in the region table of the software. For each library generated by various gDNA input amounts, the molarity was plotted in a graph comparing both systems. The data summarized in the table demonstrates that sizing and quantification of amplified libraries with the High Sensitivity D5000 ScreenTape assay match the results of the High Sensitivity DNA assay of the 2100 Bioanalyzer system.

Application note: 5991-8191EN

Next-Generation Sequencing

Quality control of final NGS libraries



Instrument: **TapeStation systems**

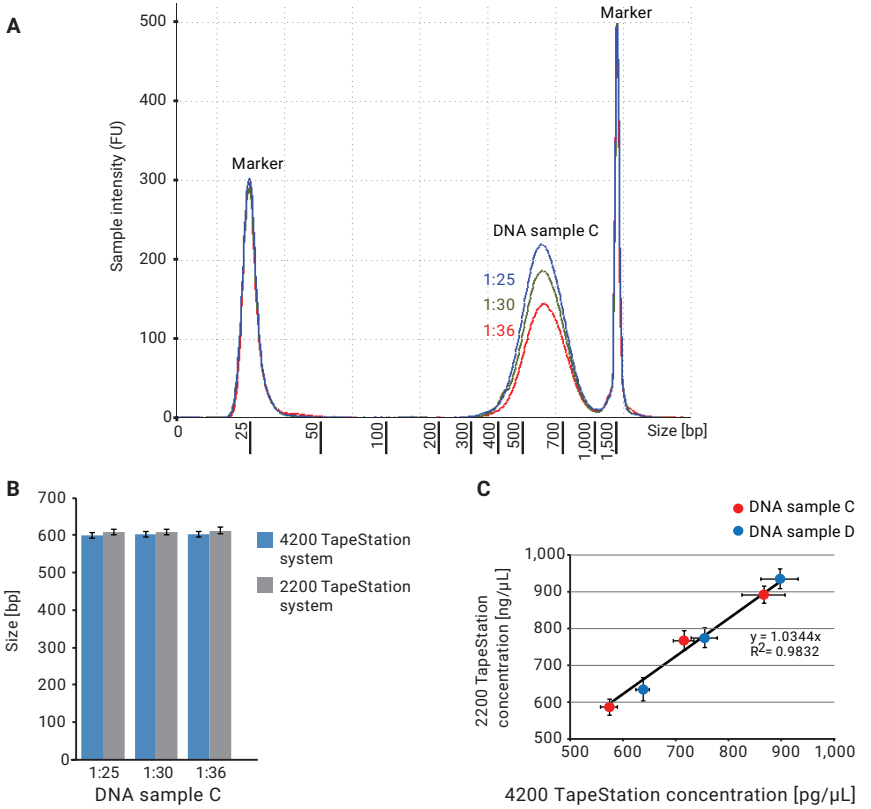
Assay: **D1000 ScreenTape assay**

Abstract: The 4200 TapeStation system was used for quality control of the final NGS libraries. These were expected to be sized between 250 and 350 bp with a minimum concentration of 2 ng/µL. In a gel view of 15 samples, lane B5 and D6 show negative controls (A). B shows an example of an electropherogram of one sample. The distribution of the concentration for all 80 samples plus eight controls is illustrated in C. The two positive controls are shown as green symbols. The red lines indicate the recommended concentration threshold (2 ng/µL). The maximum peak size for all 80 samples plus eight controls is displayed in D. The two positive controls are shown as green symbols. The red lines indicate the recommend size range (250 to 350 bp).The analysis of the final NGS libraries with the 4200 TapeStation system confirmed successful DNA library preparation for all 80 samples and the six positive control samples.

Application note: 5991-7615EN

Next-Generation Sequencing

Equivalence of the 4200 TapeStation and 2200 TapeStation systems



Instrument: **TapeStation systems**

Assay: **D1000 ScreenTape assay, High Sensitivity D1000 ScreenTape assay**

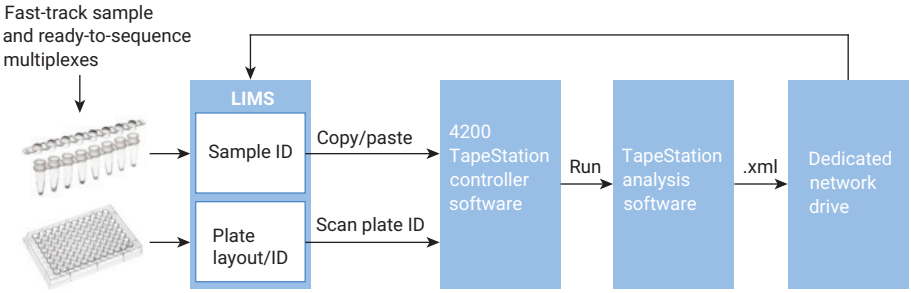
Abstract: Quality control of NGS libraries is key to the success of any sequencing run. The D1000 ScreenTape and High Sensitivity ScreenTape assays can be used for quality control, providing DNA sizing, and quantification. The bar chart shows the sizing results of a sample in 3 dilutions analyzed with the High Sensitivity D1000 ScreenTape assay on both the 2200 and 4200 TapeStation platforms. DNA concentration determined with the 4200 TapeStation system is plotted against the concentration measured with the 2200 TapeStation system for the High Sensitivity D1000 ScreenTape assay.

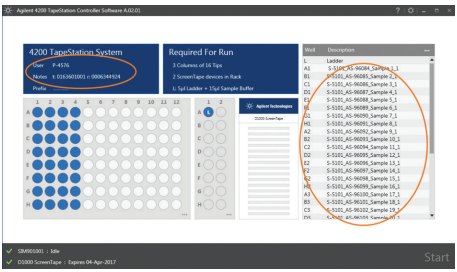
The data demonstrates that results obtained with the Agilent High Sensitivity D1000 assay, using both TapeStation systems are directly comparable and highly reproducible.

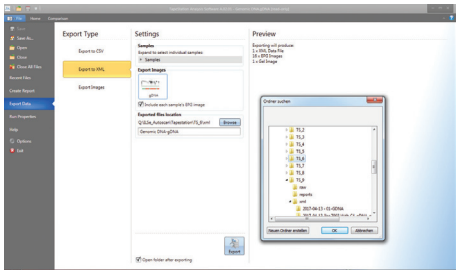
Application note: 5991-6892EN

LIMS and Robotic Integrated Systems

Integration of the 4200 TapeStation system into a laboratory information management system (LIMS)







Instrument: **TapeStation systems**

Abstract: Large core sequencing facilities implement a laboratory information management system (LIMS) to handle all sample-relevant data. This includes pre-analytical information such as sample ID and sample source, down to analytical parameters like sample concentrations, molarities, and sequencing results. The 4200 TapeStation software package allows a seamless LIMS integration. The 4200 TapeStation controller software offers different methods for importing data from a database. Sample IDs can be imported from CSV files and additional information like plate ID or lot information of reagents and ScreenTape devices can be entered into the software with an external handheld barcode scanner. Result data can be exported as an XML file and saved on a dedicated network drive. The XML file contains all relevant result data like sizing, concentration, and integrity information for analyzed DNA and RNA samples, as well as peak sizes, areas, heights, molarities, and region-specific information.

The 4200 TapeStation system offers full automation of RNA and DNA sample quality control and a reduction in manual operation work. In addition, LIMS integration further reduces manual data entry steps, resulting in increased efficiency and elimination of a potential source of errors.

Technical overview: 5991-7984EN

LIMS and Robotic Integrated Systems

Robotic integration with the 5400 Fragment Analyzer system

Instrument: **Fragment Analyzer system**

Abstract: The 5400 Fragment Analyzer system was designed for robotic integration with control through a detailed and rigorously tested API (Application Program Interface). The API is compatible with most laboratory automation systems. It controls the movement of the buffer, waste, and sample drawers, with the electrophoresis methods introduced through TCP/Ethernet or serial port connections. Users have the ability to customize API commands so they are coded to adapt to any work environment. The 5400 Fragment Analyzer instrument uses the same 96-capillary arrays as the 5300 Fragment Analyzer system, allowing for continuous separation of over 2,400 samples in 24 hours.

The integrated software package for both instrument operation and data analysis lets the user remotely control the instrument. Result files are generated and formatted to specific user criteria. Auto-data processing can be activated for large-scale assessments and sample names can be added through LIMS integrated sample data files.

Technical Information: www.agilent.com/genomics/fragment-analyzer

Overview of Featured Literature

To download an application note or to find other literature on the Bioanalyzer, Fragment Analyzer, TapeStation and Femto Pulse systems visit our website:
www.agilent.com/genomics/bioanalyzer
www.agilent.com/genomics/fragment-analyzer
www.agilent.com/genomics/tapestation
www.agilent.com/genomics/femto-pulse

Analysis of Genomic DNA	Publication Number
Application note: Quality Metrics for Nucleic Acids with the Agilent Fragment Analyzer and Femto Pulse Systems	5994-0521EN
Technical overview: High Throughput Genomic DNA Assessment by the Agilent 4200 TapeStation System	5991-6629EN
Application note: Assessment of Genomic DNA Quality with the Agilent Fragment Analyzer System	5994-0511EN
Application note: The DNA Integrity Number (DIN) Provided by the Agilent 2200 TapeStation System is an Ideal Tool to Optimize FFPE Extraction	5991-5246EN
Application note: Evaluating the Agilent 4200 TapeStation System for High Throughput Sequencing Quality Control	5991-6892EN
Application note: Analysis of High Molecular Weight Genomic DNA using the Agilent 2200 TapeStation and Genomic DNA ScreenTape	5991-1797EN
Application note: Quality Control for Agilent SureSelect QXT WGS Library Preparation	5991-8191EN
Application note: Integrating the DNA Integrity Number (DIN) to Assess Genomic DNA (gDNA) Quality Control Using the Agilent 2200 TapeStation System	5991-5442EN
Application note: Genomic DNA Sizing and Quality Control on the Agilent Femto Pulse System	5994-0516EN
Application note: Genomic DNA Extractions Compared with the Agilent Femto Pulse System	5994-0754EN
Application note: Quality Assessment of Genomic DNA for Biobanking Samples with the Agilent Femto Pulse System	5994-0520EN

Analysis of FFPE DNA

	Publication Number
Application note: DNA Quality Control of Formalin-Fixed Paraffin-Embedded and Fresh-Frozen Tissues Prior to Taget Enrichment and Next Generation Sequencing	5990-0483EN
Application note: The DNA Integrity Number (DIN) Provided by the Genomic DNA ScreenTape Assay Allows for Streamlining of NGS on FFPE Tissue Samples	5991-5360EN
Application note: Use of the Agilent 4200 TapeStation for Quality Control in the Whole Exome Sequencing Workflow at the German Cancer Research Center (DKFZ)	5991-7615EN

Analysis of Total RNA

Application note: RNA Integrity Number (RIN) – Standardization of RNA Quality Control	5989-1165EN
Application note: Quality Analysis of Eukaryotic Total RNA with the Agilent Fragment Analyzer System	5994-0519EN
Technical overview: Performance Characteristics of the RNA and the High Sensitivity RNA ScreenTape Assay for the 4150 TapeStation System	5994-1038EN
Application note: Assessing Integrity of Plant RNA with the Agilent 2100 Bioanalyzer	5990-8850EN
Application note: Assessing Integrity of Insect RNA	5991-7903EN
Technical overview: Comparison of RIN and RQN for the 2100 Bioanalyzer and the Fragment Analyzer Systems	5994-1860EN
Application note: Plant RNA Degradation Detection Using the Agilent Fragment Analyzer System	5994-0518EN
Application note: Quality Control in Illumina Sequencing Workflows Using the TapeStation System	5994-0327EN
Application note: Monitoring Library Preparation for Next-Generation Sequencing in systems Biology Omics Analysis	5994-0946EN
Application note: Evaluating the Agilent 4200 TapeStation System for High Throughput Sequencing Quality Control	5991-6892EN
Technical overview: Performance of the Agilent RNA ScreenTape and the High Sensitivity RNA ScreenTape Assay for the Agilent 2200 TapeStation System	5991-3426EN

Analysis of FFPE RNA

	Publication Number
Technical overview: Simplified DV_{200} Evaluation with the 2100 Bioanalyzer System	5991-8287EN
Application note: DV_{200} Evaluation with RNA ScreenTape Assays	5991-8355EN

Analysis of Cell-free DNA

Technical overview: Performance Characteristics of the Cell-free DNA ScreenTape Assay	5994-1390EN
Application note: Accurate QC Analysis of cfDNA Using the Agilent Fragment Analyzer System	5994-0510EN
Application note: Separation of cfDNA with the Agilent HS NGS Kit on the Agilent Fragment Analyzer System	5994-0522EN
Application note: cfDNA Separated on the Agilent Femto Pulse System	5994-0514EN

Next-Generation Sequencing

Application note: Quality control of NGS libraries with Daisy Chains	5994-2233EN
Application note: Improving Sample Quality for SureSelect Target Enrichment and Next-Generation Sequencing with the High Sensitivity DNA kit	5990-5008EN
Application note: Performance Characteristics of the High Sensitivity DNA Assay for the Agilent 2100 Bioanalyzer	5990-4417EN
Application note: Low Input DNA Size Selection on the Pippin Prep System using the Agilent 2100 Bioanalyzer with the Agilent High Sensitivity DNA Kit	5990-8382EN
Application note: Comparison of the Agilent HS Small Fragment Kit and the Agilent HS NGS Fragment Kit on the Agilent Fragment Analyzer System	5994-0515EN
Application note: Quality Control of Magnis SureSelect XT HS Workflow with Agilent Automated Electrophoresis Solutions	5994-1741EN
Application note: Monitoring Library Preparation for Next-Generation Sequencing in Systems Biology Omics Analysis	5994-0946EN
Application note: Sample Quality Control in Agilent NGS Solutions	5994-0127EN

	Publication Number
Application note: Quality Control in Illumina Sequencing Workflows Using the TapeStation System	5994-0327EN
Application note: Impact of gDNA Integrity on the Outcome of DNA Methylation Studies	5991-6427EN
Application note: Quality Control for Agilent SureSelect QXT WGS Library Preparation	5991-8191EN
Application note: Use of the Agilent 4200 TapeStation for Quality Control in the Whole Exome Sequencing Workflow at the German Cancer Research Center (DKFZ)	5991-7615EN
Application note: Evaluating the Agilent 4200 TapeStation System for High Throughput Sequencing Quality Control	5991-6892EN

LIMS and Robotic Integrated Systems

Technical overview: LIMS Integration of the Agilent 4200 TapeStation System	5991-7984EN
Robotic integration with the 5400 Fragment Analyzer www.agilent.com/genomics/fragment-analyzer	

Learn more:
www.agilent.com/genomics/automated-electrophoresis

Buy online:
www.agilent.com/chem/store

U.S. and Canada
1-800-227-9770
agilent_inquiries@agilent.com

Europe
info_agilent@agilent.com

Asia Pacific
inquiry_lsca@agilent.com

Published in the USA, July 1, 2020
PR7000-7476
5994-2142EN

Agilent
Trusted Answers